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Effects of chronic dietary inorganic arsenic in dogs

Regg Darwyn Neiger
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Effects of chronic dietary inorganic arsenic in dogs

by

Regg Darwyn Neiger

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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GENERAL INTRODUCTION

Arsenic is a non-metal or metalloid in Group V of the Periodic Table but is referred to as "arsenic metal". Predominant valences for arsenic are +3 and +5. Naturally occurring arsenic is usually pentavalent and arsenic added to the environment is trivalent (Hammond and Beliles, 1980). Arsenic combines with numerous elements in nature and is rarely present as a free element. Arsenic is ubiquitous and is present in rock, soil, water, and living organisms. Man's activities can add significant amounts of arsenic to the environment. Contamination sources include smelter and fossil fuel burning power plant stack emissions, pesticides, and cotton desiccants (Piver, 1983; Fitzgerald, 1983).

It is impossible for animals or man to avoid exposure to natural sources of arsenic. Because of arsenic's ubiquitous nature all food sources contain some arsenic. In addition to natural sources, the frequent use of arsenicals as herbicides creates the potential for accidental exposure to higher concentrations of arsenic. High level exposures result in dramatic clinical signs and high morbidity. Lesions of acute poisoning are well documented (Sullivan, 1985; Osweiler et al., 1985; NAS, 1977). Much of the information on chronic effects of arsenic exposure is from epidemiological studies in man. These studies include many uncontrolled factors that

could enhance, inhibit, or obscure the true effects of arsenic. This controlled experimental study was designed to determine at what concentration dietary arsenic causes adverse effects in dogs and what these effects are.

Numerous subchronic and chronic arsenic studies have been done with rats and a lesser number with mice (Byron et al., 1967; Schroeder and Balassa, 1967; Schroeder et al., 1968; Kiyono et al., 1974; Fowler et al., 1979b; Fowler and Woods, 1979). A need for chronic studies in higher animals exists. This is especially true since it has been found that rats metabolize arsenic differently than any other species studied (Vahter, 1983).

Only two chronic inorganic arsenic feeding studies in dogs have been done. Calvery et al. (1938) reported no clinical effects in dogs given feed containing up to 107.5 ppm arsenic as arsenic trioxide. Byron et al. (1967) fed dogs a diet containing 5.0 to 125 ppm of arsenic as sodium arsenate or sodium arsenite. The sodium arsenite high dose group lost 44 to 61 percent of its initial body weight. The sodium arsenate high dose group had less dramatic but significant weight loss. These studies left a need for a more detailed and comprehensive investigation. Close examination of feed intake, body weight, arsenic intake, and pathological changes (clinical and morphological) were not

available.

Hepatocyte ultrastructure has been morphologically evaluated in arsenic exposed rats and mice. Rats chronically exposed to sodium arsenate had significant increases in mitochondrial volume density and surface density of the inner mitochondrial membrane (Fowler et al., 1979b). Mice with a similar sodium arsenate exposure had no statistically significant hepatocyte ultrastructural changes (Fowler and Woods, 1979). No morphological study of hepatocyte ultrastructure of dogs or higher animals has been done. There is no information whether canine hepatocytic mitochondria react similarly to rat or mouse mitochondria, or differently altogether.

The objectives of this study were to:

1. Establish chronic toxic and tolerated dosages of sodium arsenite in dogs.

2. Establish the effect of dietary sodium arsenite on feed intake and body weight.

3. Evaluate hematologic, serum chemistry, and urinary effects in dogs chronically exposed to dietary sodium arsenite.

4. Morphologically and stereologically evaluate the ultrastructure of arsenic exposed canine hepatocytes.

Explanation of Dissertation Format

This dissertation is presented in the format of Toxicology and Applied Pharmacology and consists of multiple manuscripts which will be submitted to Toxicology and Applied Pharmacology.

The general introduction and literature review precede the first manuscript. A summary and discussion follows the last manuscript. A list of references appears at the end of each manuscript. Literature cited in the general introduction, literature review, and summary and discussion appears at the end of the dissertation.

The Ph.D. candidate, Regg Neiger, was the principal investigator for each study.

LITERATURE REVIEW

Chemical Nature of Arsenic

Arsenic is a member of Group V of the Periodic Table of elements. Its chemical symbol is As, atomic number 33 and atomic mass weight 74.91. There are five electrons in arsenics outer shell, two in the s orbit and three in the p orbit. Possible valences are -3, 0, +3, and +5. However the +3 and +5 are dominant. Arsenic is a non-metal or metalloid but is referred to as "arsenic metal".

It is rare to find elemental arsenic free in the natural environment. Arsenic combines with many elements besides oxygen and hydrogen including sulfur, nickel, cobalt, copper, iron, aluminum, barium, bismuth, calcium, lead, magnesium, manganese, uranium, and zinc. In one of its many forms arsenic can be found in rocks, soils, water, and living organisms in concentrations of parts per million or billion (NAS, 1977).

The National Academy of Science (1977) reviewed arsenic and its chemistry. Following is the grouping of the inorganic arsenic compounds, and their chemistry, considered of environmental importance.

Arsenic trioxide (As_2O_3) is also referred to arsenious acid anhydride or "white arsenic". It is the primary product of arsenic smelters and is formed by the roasting of metallic

arsenides of arsenic containing sulfides. It is unintentionally formed by the burning of coal containing arsenic. Arsenic trioxide is slightly soluble in water, sublimes at 135° C and melts at 272°C. This compound has direct industrial uses and is the primary ingredient in the formation of many organic and inorganic arsenicial derivatives (NAS, 1977). A few direct applications, some which are obsolete, are; insect and fungus control, glass and paint production, and timber preservation (Kipling, 1977).

Arsenic pentoxide (As_2O_5) is formed by the oxidation of elemental arsenic or arsenic trioxide by nitric acid. This compound is very soluble in water (NAS, 1977). It has been used as a chemical intermediate, defoliant and wood preservative (Kipling, 1977).

Arsenous acid (H_3AsO_3) and arsenic acid (H_3AsO_4) can be produced from arsenic trioxide being dissolved in water or nitric acid respectively (NAS, 1977).

Arsenites of the formulas MH_2AsO_3, M_2HAsO_3, and M_3AsO_3 are known. M is a univalent metal cation, or one equivalent of a multivalent cation (NAS, 1977). The character of the cation determines the solubility of the compound in water. Alkali-metal arsenites are freely soluble, alkaline-earth arsenites slightly soluble, and heavy-metal arsenites insoluble. Sodium meta-arsenite, $NaAsO_2$, can be manufactured

from arsenious oxide and caustic soda with a little water (Buchanan, 1962). Sodium meta-arsenite like other alkali-metal arsenites is highly soluble in water.

A few common arsenates are calcium arsenate [$Ca_3(AsO_4)_2$], lead arsenate [$Pb_3(AsO_4)_2$], and sodium arsenate (Na_2HAsO_4). The solubility of arsenates is dependent on the cation in the same manner as described for arsenites. Arsenates and arsenites have a wide range of uses ranging from insecticides, herbicides, wood preservatives and others.

Arsenous acid and arsenic acid esters, such as triorganyl arsenite [$(RO)_3As$] and triorganyl arsenate [$(RO)_3AsO$], can be prepared but are very easily hydrolyzed at the arsenic-oxygen-carbon bond. These esters are therefore very unstable in aqueous environments.

Arsenic acid (H_3AsO_4) and phosphoric acid (H_3PO_4) are very similar and could possibly be interchanged in some important biological molecules. Due to the rapid hydrolysis of arsenic acid esters in aqueous media, these molecules would degrade prematurely and block some important metabolic pathways. Two biologically important molecules that may be susceptible to the substitution are adenosine triphosphate and glucose-1-phosphate (NAS, 1977).

Hydrogen sulfide reacts with arsenic in anaerobic aqueous media forming arsenic sulfides which readily precipitate. Realgar (As_2S_2), orpiment (As_2S_3), and arsenic

pentasulfide are the most important arsenic sulfides. Realgar is a natural arsenic ore (NAS, 1977). Realgar and orpiment have been known since 400 B.C. and have been used as medicine, pigments, and depilatories in tanneries (Buchanan, 1962).

Sources of Arsenic

There are many ways for animals or man to be exposed to arsenic. Woolson (1983) reviewed the literature on arsenic in the environment and classified the sources of exposure as either natural or man-made. Exposures that cause clinical disease are usually due to man-made sources. Natural sources will be discussed first.

Natural sources

Earth crust The twelfth most abundant element in the crust of the earth is arsenic. The continental crust is 1.5-2.0 ug/gm arsenic. At least 245 different minerals contain arsenic. Arsenic is usually found in association with sulfur and found in the following forms (Woolson, 1983).

1) native element or alloys (4 minerals)
2) arsenides (27 minerals)
3) sulfides (13 minerals)
4) sulfosalts (sulfides of arsenic with metals, 65 minerals)

5) oxidation products of the above (2 oxides, 11 arsenites, 116 arsenates, and 7 silicates)

Of the arsenic containing minerals, arsenopyrite is the most common. When exposed to air, arsenic-containing sulfides and sulfosalts are oxidized to arsenic trioxide and then to arsenate. Rocks vary in arsenic content, sedimentary usually contain more than igneous. Sedimentary iron and manganese ores are high in arsenic with concentrations up to 2,900 and 15,000 ug/gm respectively. Oil shale averages around 100 ug/gm arsenic (Woolson, 1983). It is present at about 2 ug/gm in igneous rocks (Kipling, 1977).

Natural levels of arsenic are sometimes difficult to define. For example, the surface 6 cm of fresh water sediments is higher in arsenic than the rest. This elevation is attributed to man's activity (Woolson, 1983).

Arsenic is present in all soils with concentrations ranging between 0.1 to 40 ug/gm and averaging around 5 to 6 ug/gm. The concentration of arsenic in soil varies with the arsenic content of the rock the soil is from. The soil over sulfide ore deposits have arsenic concentrations of several hundred ug/gm. In this soil the arsenic is present as sulfide minerals, most commonly arsenopyrite, or in the inorganic anion state (Woolson, 1983). The arsenic concentration of virgin soil and forest humus is 3-5 ug/gm

(Kipling, 1977).

Arsenic bound to organic matter is freed into the soil solution by oxidation of the organic matter. Once in the soil solution the arsenic can be taken up by plants or fixed by soil cations. Plants may take up arsenic contributed to the soil solution by iron or aluminum arsenates.

The amount of arsenic available for plant uptake is a function of the chemical and physical forms of arsenic compounds present. Average available arsenic in cultivated soils is about 10 percent of the total arsenic (Woolson, 1983).

Water Arsenic is present in all water. Arsenic concentrations in water from different lakes, rivers, wells, and springs vary greatly. Most arsenic in water is from natural sources (Woolson, 1983). Most rivers and lakes have concentrations well below 0.01 mg/liter (0.01 ug/gm) but up to 1.0 mg/liter (1.0 ug/gm) (Fowler et al., 1979a). United States well water had arsenic concentrations ranging from 0.0 to 10.0 ug/gm in studies summerized by Woolson (1983). Naturally high arsenic concentrations occur in water from hot springs, ground water in areas of thermal activity, ground water of areas with high arsenic content in rocks, and water with high dissolved salt content. High arsenic levels in rivers and lakes are usually due to human sources.

A major source of human arsenic exposure is drinking

water. In the United States drinking water is responsible for an estimated daily intake of 10-12 ug of arsenic (Kipling, 1977). Only a few water supplies very high in arsenic exist. One of these is the Toconce River in Chile with arsenic concentrations up to 0.8 ug/gm. It originates in the Andes Mountians 300 kilometers above the city of Antofagasta. Antofagasta secured its water supply from the river. Chronic arsenicism caused cutaneous lesions in 30 percent of the population. Hair arsenic residues were used to support the diagnosis (Borgono and Greiber, 1971).

Forms of arsenic in natural waters are arsenate, arsenite, methylarsonic acid and dimethylarsenic acid. Methylated forms are a small percentage of the total water arsenic. The chemical form of arsenic in ground water is mostly undetermined. A sample of a few ground waters showed 25-50% of the arsenic in the trivalent form (Fowler et al., 1979a). Surface supplies of fresh water have trivalant to pentavalent arsenic ratios ranging from 6.7/1 to .002/1 in the studies summarized by Braman (1983). Inorganic trivalent and pentavalent arsenic are more of an environmental threat than organic arsenicals because they more intensely interact with the human biochemical system (Braman, 1983). It is an important fact that trivalent arsenicals are more toxic than pentavalent arsenicals.

Sea water has 0.001 to 0.005 mg of arsenic per liter (0.001-0.005 ug/gm). Arsenate has been reported as the most dominant form of arsenic in sea water (Fowler et al., 1979a). The thermodynamic calculated ratio of trivalent to pentavalent arsenic in oxygenated seawater at ph 8.1 is 0.001/1, but measured values vary from 10/1 to 0.1/1. Biologicial reduction is believed to contribute the difference between calculated and measured values. The arsenic content in sea water is a small fraction of that estimated to be carried into it (NAS, 1977). Most of the arsenic dissolved in the sea water is deposited in sediment through precipitation or adsorption on marine clays.

It is believed that surface waters, fresh and saline, are self-purifying by deposition of arsenic in the sediment. Sediments always have a higher concentration of arsenic than the water with which they are associated (NAS, 1977).

Air Arsenic concentrations in air vary from <0.02 ug/m^3 in areas remote to industry to 0.16 ug/m^3 in some urban areas (NAS, 1977). A quarterly average of 1.4 ug/m^3 was recorded in El Paso, Texas near a large copper smelter (Fowler et al., 1979a).

Plants Arsenic is ubiquitous in the plant kingdom. The concentrations in plants vary from <0.01 to 5 ug/gm on a dry-weight basis. The concentration of naturally occurring arsenic in plants depends on variation between individual

plants, plant species, available soil arsenic, and growing conditions (NAS, 1977). Natural plant arsenic accumulation is almost always in the pentavalent form (Kipling, 1977). Injury occurs to plants accumulating arsenic before plant arsenic concentrations are toxic to animals that may eat them (NAS, 1977). However, arsenical insecticide or herbicide surface contamination increases arsenic concentrations in plants to the point of causing a hazard.

Marine plants have a high naturally accumulated arsenic content. British seaweed has arsenic concentrations ranging from 5.2 to 94 ug/gm (NAS, 1977).

Animals Arsenic is present in all living organisms. Domestic animals and man usually have less than 0.3 ug/gm on a dry matter basis. Fresh water fish have concentrations usually less than 1 ug/gm and marine fish up to 10 ug/gm. Bottom dwelling and feeding marine organisms generally have high arsenic concentrations ranging up to 100 ug/gm (NAS, 1977).

Foodstuffs As noted above all living organisms contain arsenic and therefore all foodstuffs must contain arsenic. In the United States a specific non-seafood diet gives a person 0.04 mg of arsenic per day. A more average diet gives about 0.19 mg of arsenic per person per day. In Japan, the daily arsenic intake is 0.07-0.37 mg/person.

Japan has a higher arsenic intake probably due to increased consumption of food from the sea. Marine organisms contain about ten times as much arsenic as other food sources (Fowler et al., 1979a).

Man-made sources

This section will deal with the sources of arsenic that are the result of the perturbation of the natural distribution of arsenic by man.

Man realized the value of arsenic compounds in very early times. Arsenic sulfides were known to and recommended by Hippocrates (460-377 B.C.) for medicinal uses (Buchanan, 1962). The listing of arsenical compounds and their uses through the ages would be interesting but overwhelming. The following discussion will be limited to major uses in recent times.

Sources from the utilization of natural resources

Mining and processing of natural resources releases arsenic into air, water, and soil. A few important examples are discussed below.

Most mining operations produce large amounts of tailings dumped in piles next to the mine or processing plants. The sulfur compounds in the tailings oxidize and combined with water to form acid. The acid solution dissolves and leaches away many elements including arsenic. Therefore the drainage

waters of these piles can be high in arsenic. The effect on the environment depends on the accessibility of the drainage water to natural surface or ground water (Piver, 1983).

Lagoons of water for processing ore or coals can be high in arsenic. Contaminated soil and ground water can occur if a low permeability lagoon liner is not present (Piver, 1983).

Smelters can be major point sources of arsenic. Arsenic trioxide is abundant in crude flue dust from lead, copper, and gold smelters. Collecting and roasting this dust is how most commercial arsenic is obtained. Efforts are made, with variable success, to prevent the emission of the flue dust into the environment. Despite these efforts a portion of the arsenic trioxide is emitted as particulates into the air (Piver, 1983).

A well documented point source of arsenic is the copper smelter in Tacoma, Washington. The Puget Sound Air Pollution Control Agency estimates the yearly arsenic trioxide emission into the air was 1,176 tons in 1971 decreasing to 400 tons in 1980 (Piver, 1983). Air concentrations were 0.5 to 2.5 ug/m^3 at the property line and 0.02 to 0.13 ug/m^3 eight miles from the smelter. Settling and rainfall increased the soil arsenic concentrations in the area. Soil concentrations were 380 ug/gm near the smelter, and 100-300 ug/gm on islands four kilometers to the north by north east. Arsenic concentrations in dust from houses in the vicinity were 77 to

4,461 ug/gm (Woolson, 1983). Background soil concentrations must be considered when interpreting these figures because smelters are probably located in arsenic rich areas.

Fossil fuel combustion releases arsenic into the air. Eastern, mid-western, and western United States coal contains an estimated 10, 5, and 1 ug/gm of arsenic respectively. Most of the arsenic is trapped in the dust collecting equipment of coal burning plants.

An estimated eight hundred forty tons of arsenic was collectively emitted from coal burning plants in 1973 (Woolson, 1983). Therefore coal burning does not have as much effect on local environmental arsenic concentrations as a smelter does.

The average arsenic concentration of oil is 0.042 ug/gm. Arsenic concentrations in emissions from oil burning plants should not cause an environmental problem. Shale oil on the other hand could cause a problem. Oil from Colorado shale has an arsenic concentration of 82 ug/gm (Woolson, 1983).

Manufactured sources United States consumption of arsenic has been going down since the sixties and early seventies. However the consumption of arsenic trioxide, the raw material of most arsenicals, in the late seventies was still about twenty thousand short tons (Fitzgerald, 1983). The American Smelting and Refining Company estimated the 1981

United States arsenic market to be as follows.

Wood Preservatives	36%
Herbicides	31%
Cotton Desiccants	15%
Moly Flotation	8%
Glass Production	5%
Miscellaneous	5%

Following is a list of a few common arsenicals and their uses.

Arsenic acid	Desiccant (cotton) Wood preservative
Chromated copper arsenate	Wood preservative
Arsenic trioxide	Rodenticide
Calcium arsenate	Pesticide
Lead arsenate	Grapefruit growth regulator
Sodium arsenate	Ant killer
Sodium arsenite	Herbicide
Methane arsonic acid & salts	Herbicide
Cacodylic acid & sodium salts	Defoliator (cotton)
Arsanilic acid	Feed additive
3-nitro-4-hydroxyphenylarsonic acid	Feed additive

Exposure to these chemicals can occur during their production, distribution, or application. Their application can produce residues in the air, soil, water, plants, or

animals which are also sources of exposure.

Repeated high level use of arsenical pesticides raises the arsenic content of soil significantly. Woolson (1983) summarized a number of studies on the effect of repeated use of lead arsenate, calcium arsenate or sodium arsenite on soil residues. Most residues were in the area of 100 to 200 ug/gm compared to background levels from 5 to 15 ug/gm.

In orchards where inorganic arsenical pesticides were used at high levels for prolonged periods the soil becomes phytotoxic. Plant growth is limited in soils high in arsenic; therefore significant human exposure to arsenic by comsumption of plants grown in these soils is minimal (NAS, 1977).

Woolson (1983) also reviewed literature on various arsenical pesticides experimentally applied. He concluded that if the chemicals were used at currently recommended levels there was minimal accumulation.

The use of chromated copper arsenate and ammoniacal copper arsenate as wood preservatives is increasing greatly. One hundred fifty million cubic feet of wood was treated with these products in 1979, versus fifteen million cubic feet in 1969 (Baldwin, 1983). Wood treated this way only minimally contaminates soil with which it is in contact (NAS, 1977).

Feed additive arsenicals do not greatly increase arsenic levels in animal products over background levels. In fact

most normal marine animal arsenic levels are higher than those of domestic animals treated with arsenical feed additives (NAS, 1977).

Inorganic Arsenic as a Toxicant

History

I am an evil, poisonous smoke...
But when from poison I am freed,
Through art and sleight of hand
Then can I cure both man and beast,
From dire disease ofttimes direct them;
But prepare me correctly, and take great care
That you faithfully keep watchful guard over me;
For else I am poison, and poison remain,
That pierces the heart of many a one.

Valentini, 1694 (NAS, 1977)

This poem exposes the double edged nature of arsenic. By 2000 B.C. arsenic trioxide, produced as a byproduct of copper smelting, was used as a medicine and a poison (Pershagen, 1983).

Professional poisoners used arsenic extensively in the Middle Ages. In the nineteenth century one third of the criminal poisonings in France were blamed on arsenic (Pershagen, 1983).

In the 1700s and 1800s Fowler's solution, a one percent

solution of arsenic trioxide, was used extensively for diseases such as psoriasis and bronchial asthma. The mild toxic side effects of gasterointestinal discomfort and pain were ignored. If the oral dose could not be tolerated, per rectum administration was used. If the treatment caused severe signs of vomiting and diarrhea the dose was lowered (Pershagen, 1983).

Occupational toxic exposures increased with the increased industrial use of arsenic over the last 100 years.

Well documented mass human poisonings have occurred through contamination of food and drink with arsenic. The Manchester (England) beer incident in 1900, the Morinaga (Japan) milk incident of 1955 and the soy sauce incident of 1956 in Japan are well documented cases (Pershagen, 1983).

The accidental contamination of the environment from the utilization of natural resources and the continued industrial, agricultural and domestic use of arsenic make the possibility of poisoning real.

Metabolism

Absorption Studies in mice, dogs, and monkeys have shown that aqueous solutions of inorganic arsenicals are almost completely absorbed from the gastrointestinal tract (Charbonneau et al., 1978a; Hollins et al., 1979; Vahter and Norin, 1980). Mice absorb trivalent and pentavalent

inorganic arsenic solutions at the same rate. The absorption was not dose dependent within the range 0.4 to 4.0 mg/kg of bodyweight (Vahter and Norin, 1980).

If a toxicant is relatively insoluble it will have limited contact with the gastrointestinal mucosa and will not be extensively absorbed. Limited gastrointestial absorption occurs if a powdered arsenical particle size is too large (Klaassen, 1980). Lack of absorption is why insoluble arsenical compounds or arsenicals consisting of coarse particles are less toxic than highly soluble arsenicals (Schwartze, 1922; Done and Peart, 1971).

Topically applied inorganic arsenicals not only cause local skin damage but are absorbed causing systemic poisoning (Buchanan, 1962; Evinger and Blakemore, 1984).

Respiratory absorption depends on the chemical form of the inorganic arsenic inhaled (Pershagen, 1980). Water soluble sodium arsenate is rapidly absorbed from the lungs of rats (Dutkiewicz, 1977).

Tissue distribution

Blood After absorption, arsenic is transported in the blood to all organs of the body. Clearance of arsenic from the blood of humans, dogs, mice, and rabbits follows a two or three phase exponential curve (Ducoff et al., 1948; Charbonneau et al., 1978b; Vahter and Norin, 1980; Bertolero

et al, 1981). The first phase is the largest (>90%). It is eliminated rapidly with a half-time of one to two hours. The second and third phases have estimated half-times of 30 and 200 hours respectively.

Rats are significantly different than the above mentioned species. Shortly after dosing they have high blood arsenic concentrations that remain high (Ducoff et al., 1948). Blood half-times are over 60 days (Lanz et al., 1950). This is due to arsenic binding to the eyrthrocytes. Blood arsenic concentration is 10 to 100 times higher than most other tissues (Klaassen, 1974)

Arsenic in rabbit and rat erythrocyte cytosol is associated with high molecular weight proteins (Marafante et al., 1982). Arsenic binds to erythrocyes longer and in higher concentrations in rats than rabbits. The concentration of arsenic in the red blood cells relative to the plasma varies with the valence of the arsenical. The trivalent inorganic arsenicals bind erythrocyte cytosol more readily than the pentavalent inorganic arsenicals (Vahter and Norin, 1980).

In rabbit studies, inorganic arsenic is poorly bound to plasma proteins (Bertolero et al., 1981; Marafante et al., 1981). After administration of trivalent or pentavalent inorganic arsenic the forms found in the blood are: trivalent and pentavalent inorganic arsenic, dimethylarsinic

acid (DMA), and methylarsonic acid (MMA).

Other tissues The main organs of accumulation of inorganic arsenic in rabbits and mice are liver, kidney, and intestinal mucosa (Deak et al., 1976; Vahter and Norin, 1980). Human distribution is similar (Ducoff et al., 1948). Parenteral administration results in rapid high intestinal mucosal arsenic concentration (Deak et al., 1976; Lindgren et al., 1982). Vahter (1983) believes the accumulation is caused by blood transport versus bile excretion due to its rapid occurrence.

Fibroblasts in cell culture accumulate greater concentrations of trivalent arsenic than pentavalent (Fischer et al., 1985). Mice given a dose of 4 mg/kg accumulate higher tissue arsenic concentrations if the arsenic given is in the form of arsenite versus arsenate (Vahter and Norin, 1980). This is also true in rabbits (Vahter and Marafante, 1983). At lower doses this difference is not as large. The difference is more apparent if the route of administration is intra-venous versus oral. The bone is different than most tissues having higher affinity for arsenate than arsenite (Deak et al., 1976; Lindgren et al., 1982).

When low doses of arsenate or arsenite are given, parentally or especially orally, the differences in tissue arsenic concentrations are small or insignificant (Sabbioni

et al., 1979; Vahter and Norin, 1980). A likely explanation is that at low concentrations the body can readily methylate both arsenate and arsenite completely to dimethylarsinic acid therefore negating any difference in valence. At high doses the body is overwhelmed and a large percentage of the dose remains in the trivalent or pentavalent form and tissue concentration differences occur. Affinity of trivalent arsenic for protein sulfhydryl groups is thought to cause the observed high tissue concentrations.

Placental transfer of arsenic has been shown in hamsters, mice, and monkeys (Hanlon and Ferm, 1977; Lindgren et al., 1982).

Two to three days after a one time exposure to inorganic arsenic, concentration of arsenic in most organs falls off rapidly, the exception being erythrocytes in rats.

The lens of the eye, hair, and nails are the last organs to give up their arsenic (Vahter, 1983).

When experimental animals are exposed to arsenic in drinking water or by inhalation, tissue concentrations increase for 2 weeks and then decrease despite continued arsenic exposure (Vahter, 1983). Possible explanations are increased excretion capacity, or decreased absorption. Increased methylation of arsenic would increase its urinary excretion rate. Another possible explanation has been worked out in rats. Orally administered arsenite binds to

glutathione in the intestinal mucosa of rats and increases mucosal cell synthesis of glutathione to twice normal concentrations (Pisciotto and Graziano, 1980). This prevents the systemic distribution of the arsenic.

Biotransformation of inorganic arsenic Two to 3 days after exposure to inorganic arsenic humans, mice, rabbits, and dogs excrete 46, 83, 83,and >95 percent of the administered arsenic in the urine respectively (Buchet et al., 1981; Marafante et al., 1981; Tsukamoto et al., 1983b; and Vahter et al., 1984). Urinary arsenic is present as inorganic arsenic, MMA, or DMA. Mice and rabbits differ from humans in that they excrete very little MMA. Humans excrete 20 to 30 percent of a total dose of arsenic as MMA in the urine (Vahter, 1983). In fibroblast cell cultures both trivalent and pentavalent inorganic arsenic are converted intracellularly to MMA and DMA and released into the culture medium (Fischer et al., 1985). Cell uptake and methylation of trivalent arsenicals is greater than that of pentavalent arsenicals.

When trivalent inorganic asenic, rather than pentavalent, is given to a rabbit or mouse, an increased percentage of the excreted urine arsenic is in the DMA form and less in the inorganic form (Vahter, 1983). Over 50 percent of the total arsenic in ultrafiltrable fractions of

liver and kidney of rabbits exposed to 0.001 mg/kg of arsenite is in the DMA form (Bertolero et al., 1981).

Recent studies suggest the liver as the major site of arsenic methylation (Buchet and Lauwerys, 1985; Marafante et al., 1985). Homogenates of rat eyrthrocytes, brain, lung, intestine, and kidney had insignificant inorganic arsenic methylating capacity compared to the liver. The proposed detoxification of inorganic arsenic goes as follows; the pentavalent form is reduced to the trivalent form, then a methyl group is added to form MMA and a second methyl group added to MMA to form DMA.

Mice and rabbits given pentavalent arsenate have trivalent arsenic in their plasma and urine after one hour (Vahter and Envall, 1983).

DMA injected into mice and rabbits is essentially completely excreted in the urine within 24 hours (Vahter and Envall, 1983). This is evidence that DMA production is an important step in the elimination of arsenic from the body.

In vitro arsenic methylation in rat liver occurs in the cytosol and accepts only trivalent arsenic as a substrate. Glutathione is required and s-adenosylmethionine is the essential methyl donor (Buchet and Lauwerys, 1985).

Dose level affects in vivo methylation of arsenic. The percent of the total urine arsenic in the form DMA goes down as the dose goes up (Vahter, 1983).

In mice a higher degree of methylation occurs if arsenic is given in the form of arsenite rather than arsenate (Vahter, 1981). Subcutaneous administration of arsenite to mice gives less methylated urinary arsenic and higher tissue retention rates than oral administration (Vahter, 1981). Pretreatment of fibroblast cell cultures with low doses of trivalent arsenic caused increased biotransformation of inorganic arsenic to MMA and DMA (Fischer et al., 1985). This made the fibroblasts less sensitive to toxic doses of trivalent or pentavalent inorganic arsenic.

Elimination

Fecal elimination Usually little arsenic is eliminated via the feces when a soluble inorganic arsenical is orally administered. That is if the preparation is a solution or a powder of fine particle size.

Biliary excretion is highly significant in some species. Rats excrete arsenic in the bile 40 times faster than rabbits and 800 times faster than dogs (Klaassen, 1974). Biliary excretion is higher if the arsenical is in the trivalent versus the pentavalent form (Vahter and Norin, 1980). Enterohepatic circulation causes little biliary arsenic to be eliminated in the feces, even when the biliary excretion is large (Klaassen, 1974).

Urinary elimination In most species 40 to 70 percent of an absorbed dose of inorganic arsenic is excreted in the urine within 48 hours (Vahter, 1983). The rat is a major exception to this rule.

Urinary excretion rates of arsenic are affected by valence, methylation, dose, route of administration, and animal species (Vahter, 1983). Trivalent arsenic is excreted slower from mice than pentavalent arsenic even though trivalent arsenic is largely methylated. This is due to the binding of trivalent arsenic to tissues and the excretion of pentavalent arsenic into the urine without methylation (Vahter, 1981; Vahter and Envall, 1983; Vahter and Marafante, 1983).

The more efficient the methylation process the more efficient the urinary excretion. In vivo inhibition of methyltransferase activity causes decreased urinary excretion and increased tissue concentrations of arsenic after the administration of arsenate or arsenite (Marafante and Vahter, 1984; Marafante et al., 1985). Methylation and urinary excretion are decreased by parenteral administration and an increased dose of arsenic (Vahter, 1981). Species difference in urinary excretion may be due to different abilities to methylate arsenic (Vahter, 1983).

Other routes of elimination Sweat, hair, milk, skin desquamation, and exhalation are arsenic elimination routes of low significance relative to urinary excretion.

Biological half-time A three-exponential function best fits the whole body elimination of inoganic arsenic in man and dog (Hollins et al., 1979; Pomroy et al., 1980). The half-times of the first two compartments are significantly smaller in the dog. This is probably due to more efficient methylation of arsenic in dog than man.

Factors effecting toxicity

Purity The greater the purity of an inorganic arsenic preparation the greater its toxicity. Harrisson et al. (1958) showed this to be true in mice and rats orally exposed to solutions and dry preparations of arsenic trioxide. This means if 99 and 95 percent pure preparations were given to an animal on an equal arsenic basis, the 99 percent preparation would be the most toxic even when the total arsenic given was the same. Crude preparations of arsenic trioxide were greater gastrointestinal irritants but less lethal than preparations of greater purity.

Solubility Solutions of inorganic arsenicals are much more toxic than powders of the same compound (Harrisson et al., 1958). As a general rule the more soluble the arsenical compound the greater its toxicity (Done and Peart,

1971).

Particle size The smaller the particle size of a dry inorganic arsenical, given orally, the greater its toxic effect (Schwartze, 1922).

Valence Trivalent arsenicals are 4 to 10 times more toxic than pentavalent arsenicals. This has been shown to be true in in vivo and in vitro (tissue culture) studies (Franke and Moxon, 1936; Savchuck et al., 1960).

Trivalent arsenicals interact with sulfhydryl groups of compounds in biological systems. In many cases this alters the activity of vital compounds and causes toxicosis. Most documentation is on inactivation of enzymes but coenzymes and substrates could also be effected (Squibb and Fowler, 1983). Long lists of enzymes susceptable to trivalent arsenic have been compiled (Webb, 1966; Squibb and Fowler, 1983).

Pentavalent arsenicals are reduced to more toxic trivalent forms in vivo. But pentavalent arsenic ion (arsenate) can also cause direct toxic effects. Squibb and Fowler (1983) have summarized the literature which shows that arsenate ion is isosteric and isoelectric with phosphate ion. Therefore, arsenate can substitute for phosphate in many important metabolic reactions. The arsenate esters formed undergo instantaneous spontaneous hydrolysis termed "arsenolysis". This causes interruption of metabolic pathways by spontaneous breakdown of critical intermediates

such as glucose-6-arsenate. Also cell energy stores can be depleted by the production of ADP-arsenate, instead of ATP, that spontaneously hydrolyzes producing no work (Moore et al., 1983; Squibb and Fowler, 1983).

Species Susceptibility to inorganic arsenic varies between species as follows: man> dog> rat> mouse (Harrisson et al., 1958; Hayes, 1982). Dangerous consequences can result from extrapolating toxicity data from rat and mouse studies for use in human situations.

Much of the experimental data on arsenicals is based on rat studies. It is generally accepted now that the rat is a poor animal model due to its unique metabolism of arsenic. A summary of the literature by M. Vahter (1983) shows that the blood half-life in the rat is months verses hours for humans, dogs, mice, and rabbits. This is due to binding of arsenic to rat erythrocytes. The binding of arsenic to erythrocytes causes rats to excrete arsenic very slowly into the urine and feces. The arsenic is rapidly excreted into the bile for the first six minutes after dosing. Then bile excretion rapidly decreases due to the redistribution of the arsenic from the liver to the blood. However, less than 10 percent of the total dose is excreted into the feces in the first seven days due to enterohepatic circulation. Only 20 percent of the total dose is excreted by the urine and feces in seven days

(Klaassen, 1974).

Condition of the animal Weak, debilitated, and dehydrated animals are more susceptible to arsenic toxicosis than normal healthy animals (Osweiler et al., 1985).

Toxicosis

Inorganic and trivalent aliphatic arsenicals cause similar toxicoses (NAS, 1977). Phenylarsonic acid (aromatic) compounds cause different clinical signs than inorganic arsenicals. These aromatic arsenicals cause incoordination and paralysis due to peripheral neuropathy (NAS, 1977). Discussion of inorganic arsenical poisoning will be divided into two parts, acute to subacute and subchronic to chronic. Descriptions that follow are of toxicoses produced by oral exposure unless otherwise noted. In general, signs and lesions are similar when arsenic is administered orally or parenterally.

Acute to subacute toxicosis Experimental studies verify descriptions of spontaneous poisonings. Peracute and acute oral poisonings have dramatic signs with high morbidity and mortality. Signs may occur within minutes if a high dose of dissolved arsenic is ingested. Death may occur in hours. Progression of signs is as follows: intense abdominal pain, salivation, vomiting, staggering gait and weakness, diarrhea, rapid weak pulse, prostration, subnormal

temperature, collapse, and death (NAS, 1977; Osweiler et al., 1985)

The course of subacute oral poisoning is basically the same as acute but slightly prolonged. Death occurs in several days. This is long enough for anorexia and oliguria to develop and be assessed. The urine contains protein, red blood cells, and casts (NAS, 1977; Osweiler et al., 1985).

Peracute poisoning causes death so rapidly no gross or microscopic lesions may be present. Gross lesions of acute and subacute poisoning consist of multifocal to diffuse reddening of the mucosa of the stomach and proximal small intestine, variable amounts of watery fluid in the gastrointestinal tract, a soft yellow liver, and wet red lungs. If the animal lives longer then edema, hemorrhage, and necrosis of the gastrointestinal tract cause blood and shreds of mucosa to be present in the stool. Perforation of the stomach or intestine may occur. There is splanchnic organ congestion with petechial hemorrhage of serous membranes. Hemorrhages are especially prominent in the heart (NAS, 1977; Sullivan, 1985). Gastrointestinal lesions are the most consistent and prominent of those listed. The others may not be present.

Histological lesions consist of: gastrointestinal mucosal and submucosal congestion, edema, and variable hemorrhage; gastrointestinal mucosal epithelial necrosis and

sloughing; mild fatty degeneration to necrosis of liver; renal tubular degeneration; and if the animal lives three to four days, cerebral edema and petechiation (NAS, 1977; Osweiler et al., 1985; Sullivan, 1985).

Harrisson et al. (1958) showed that in mice and rats solutions of crude arsenic trioxide caused marked gastrointestinal hemorrhage whereas solutions of pure arsenic trioxide only caused slight reddening. Percentages of purity of the two preparations were 97.7 and 99.9 respectively. The impurities may in themselves be gastrointestinal irritants.

Frank liver necrosis is not common but has been reported in rabbits exposed to inorganic arsenicals and dogs exposed to arsphenamine (an aromatic organic arsenical) (Soffer et al., 1937; Von Glahn et al., 1938).

Renal tubular degeneration is reported by many authors as a common lesion produced by inorganic arsenicals. Tsukamoto et al. (1983a) reported renal tubular degeneration and necrosis in dogs exposed to intravenous sodium arsenate.

If the poisoning occurs by skin contact, many of the same systemic signs described above occur. However, in this case skin lesions are prominent with blistering, edema, cracking, bleeding, and secondary infection (NAS, 1977; Evinger and Blakemore, 1984).

Inorganic arsenic is often considered a "capillary

poison". The acute toxic effects are attributed to a direct action on capillaries. This conclusion is supported by the observed congestion, edema, and hemorrhage present in visceral organs of acute poisonings. Webb (1966) reviewed the literature and found considerable contradiction on the effect of arsenicals on capillaries. He recommended that more thorough and critical work be done before definite statements could be made. Review of recent literature does not provide experimental verification that arsenic is a capillary poison. It is clear splanchnic capillary networks are affected by arsenic (Hanna nd McHugo, 1960). But whether this is a direct or indirect effect of arsenic is far from clear.

Subchronic to chronic toxicosis There is little documentation of spontaneous chronic arsenic poisoning in animals. However, the human medical literature is filled with descriptions of human populations chronically exposed to arsenic. The descriptions vary considerably and may even conflict, reflecting the variation in exposure scenarios. Pershagen reviewed numerous epidemiologic studies of chronic human exposure to arsenic. Major changes cited were: hyperpigmentation, palmoplantar hyperkeratosis, basal cell carcinoma, and squamous cell carcinoma of the skin; rhinopharyngolaryngitis; nasal septum perforation; bronchitis; emphysema; lung cancer; portal hypertension,

cirrhosis, and angiosarcoma in the liver; cardiovascular disease; anemia; granulocytopenia; lymphocyte chromosome abnormalities; neoplasia of the hematopoietic and lymphatic systems; peripheral neuropathy; encephalopathy; congenital defects; spontaneous abortion (Pershagen, 1983).

Numerous controlled experiments have been done on the effects of subchronic and chronic exposure to inorganic arsenic. Following is a summary of some of these effects.

Weight gain and feed consumption are adversely affected in a dose dependent manner. Weanling piglets had reduced weight gain and feed consumption when fed feed containing 100 ppm of sodium arsenite and 0.175 ppm selenium (Morrison and Chavez, 1983). The effect was negated if the arsenite contaminated feed was deficient in selenium (0.075 ppm) therefore suggesting that selenium enhances the toxic effect of arsenic. This contradicts the widely held belief that arsenic and selenium are antagonistic.

Mice were exposed for six weeks to drinking water containing 0.0, 20, 40, and 85 ppm of arsenic as sodium arsenate. The 40 and 85 ppm groups had a significant depression of weight gain and water consumption (Fowler and Woods, 1979).

Growth rates of mice and rats given 5.0 ppm of sodium arsenite in the water were not affected. The exposure lasted

from weaning to natural death. Life-spans of the exposed mice, but not rats, were somewhat shortened (Schroeder and Balassa, 1967; Schroeder et al., 1968). Rats exposed for 6 weeks to 215 ppm of arsenic as arsenic trioxide in the feed showed no adverse effects (Finner and Calvery, 1933). An aqueous solution of 5.0 to 10.0 mg of arsenic trioxide was given orally to infant rats on day 1 to day 21 of life. Exposed rats had a dose dependent decrease in weight gains (Kiyono et al., 1974). Rats exposed to 85 ppm arsenic as sodium arsenate in the drinking water had a significant depression of growth rate (Fowler et al., 1977).

In another study, rats were given feed contaminated with 250, 125, 62.5, 31.25, 15.63, or 0 ppm of sodium arsenite or 400, 250, 125, 62.5, 31.25, or 0 ppm of sodium arsenate. Both compounds caused a dose dependent depression of body weights. Weights of the lower dose groups were not affected. Survival rates were decreased in the high dose group fed sodium arsenite and in the two highest dose groups fed sodium arsenate (Byron et al., 1967).

Dogs chronically fed 0.5 or 2.0 mg of arsenic per Kg of body weight daily had no adverse effects. The arsenic was in the form of finely powdered arsenic trioxide. Daily feed consumption and weekly body weight were among the parameters measured (Calvery et al., 1938).

In a large two year study, 10 groups of six dogs each

were fed either sodium arsenite or sodium arsenate at arsenic levels of 125, 50, 25, 5, or 0 ppm. Five of six dogs in the 125 ppm sodium arsenite group were found dead or moribund within nine months. The last dog in that group died at 19 months into the experiment. These dogs lost 44 to 61 percent of their initial body weight. A dog on 5 ppm of sodium arsenite and one control dog also had severe weight loss. Emaciation was the only consistent post-mortem finding of the above mentioned dogs. Weight loss was significant in the 125 ppm sodium arsenate group but only one of these dogs died before the end of the test. High dose groups on both compounds developed mild anemia (Byron et al., 1967).

Inorganic arsenic is accumulated in the livers of exposed animals. Arsenic has been associated with liver damage throughout history and has been the subject of many studies.

Sixteen rabbits weighing between 1,250 and 1,500 grams were fed oats and hay daily contaminated by 1.5 mg of arsenic as sodium arsenate. Rabbits were periodically terminated from day 3 to day 231. Histopathology of the livers showed early hepatocyte necrosis which was most extensive in the portal areas. In time the necrosis was replaced by fibrosis, causing marked widening of the portal areas. Bile duct proliferation was present in many animals but not always. All exposed animals had some fibrotic changes except the one

terminated on day three, which had necrotic changes (Von Glahn et al., 1938).

Rats consuming 500 gm of feed containing 215 ppm of arsenic as arsenic trioxide had no gross or microscopic liver changes. Six weeks was the average time needed for each rat to consume 500 mg of feed (Finner and Calvery, 1939). Another author reported mild hepatocyte swelling in rats given feed containing 50 ppm of arsenic as sodium arsenate for 10 weeks (Mahaffey et al., 1981). The later study showed a significant unexplained decrease of serum alkaline phosphatase and serum glutamate-oxalate transaminase.

More than 80 percent of rats fed inorganic arsenic for two years had common bile duct enlargement. Feed contained 250 ppm of arsenic as sodium arsenite or 400 ppm of arsenic as sodium arsenate. Incidence of the lesion decreased as the arsenic concentration decreased. Other treatment changes reported from this study included "some" proliferation of small intrahepatic bile ducts in the high dose groups of either compound and variation of hepatocyte size in high dose sodium arsenite groups (Byron et al., 1967).

Hepatoctye mitochondria of rats exposed to sodium arsenate for six weeks in the drinking water underwent morphological and functional change. Significant increases occured in the mitochondrial volume density and surface

density of the inner mitochondrial membrane. Increased in vivo incorporation of ^{14}C-leucine into mitochondrial proteins were present in arsenic treated rats. Cytochrome C oxidase and monoamine oxidase are inner and outer mitochondrial membrane enzymes respectively. Activities of both of these enzymes were increased. The activity of malate dehydrogenase, a mitochondrial matrix enzyme, was not affected. Mitochondria, using pyruvate-malate as a substrate, had decreased state 3 respiration and a decreased ratio between state 3 and state 4 respiration. The mitochondrial NAD/NADH ratio was double. Respiration with succinate as the substrate was not affected except at high doses of sodium arsenate (Fowler et al., 1977; Fowler et al., 1979b). Mice given sodium arsenate at this same rate did not have significant mitochondrial morphological changes. The biochemical functional changes were not present or were present only to a lesser extent (Fowler and Woods, 1979).

In a two year study ten groups of six month old beagles were fed 0.0, 5.0, 25, 50, 125 ppm arsenic as sodium arsenite or sodium arsenate. All of the high dose group dogs for both compounds had iron-positive brown granular pigment in liver macrophages. The incidence of the pigment was low in lower dose groups. Otherwise, all the livers were grossly and microscopically unremarkable (Byron et al., 1967).

Urine is the major route of excretion of inorganic

arsenicals. This causes the kidney to be exposed to large amounts of arsenic in exposed animals. Acute parenteral administration of sodium arsenate in dogs or p-nitrophenylarsonic acid in rats produces renal tubular damage (Price, 1979; Tsukamoto et al., 1983a).

Renal changes are not dramatic in animals chronically exposed to inorganic arsenic. No or only subtle light microscopic renal lesions were reported in numerous studies of rats chronically exposed to inorganic arsenic in the feed or drinking water (Finner and Calvery, 1939; Byron et al., 1967; Schroeder et al., 1968)

Rats were exposed to drinking water containing 40, 85, or 125 ppm arsenic as soduim arsenate for six weeks. Renal cortical mitochondria of the 85 and 125 ppm groups had decreased state three respiration and respiratory control ratios (state three respiration/state four respiration). All treated groups had swollen mitochondria and increased numbers of autophagolysosomes in renal proximal tubule cells (Brown et al., 1976). Rats in another study were given feed containing 50 ppm arsenic as sodium arsenate for ten weeks. These rats also had moderate mitochondrial swelling in renal proximal tubule cells. A four fold increase of renal copper concentration was present in exposed rats. The author raises the question of the role copper may have in mitochondrial

alterations (Mahaffey et al., 1981).

Dogs were given feed containing 5 to 125 ppm of arsenic as sodium arsenite or sodium arsenate. After two years of exposure there were no gross or light microscopic renal lesions (Byron et al., 1967).

Anemia has been reported in cases of arsenic intoxication in man (Pershagen, 1983). Dogs chronically exposed to 125 ppm of sodium arsenate or sodium arsenite in the feed were mildly anemic. However, these dogs were emaciated which could account for the anemia (Byron et al., 1967). Erythroid maturation inhibition was produced in mice by intraveneous injection of sodium arsenite or sodium arsenate (Morse et al., 1980). This suggests a direct effect of arsenic on the erythron.

Squibb and Fowler (1983) reviewed a number of studies on the effect of inorganic arsenic on the immune system. They found arsenic could enhance or inhibit immunity depending on the dose of arsenic and immune component measured.

Mice were more susceptible to a viral pathogen when they were exposed to arsenic. Follow up studies showed arsenic inhibited induction and function of interferon in cell cultures. However, low doses of arsenic in mouse embryo cells increased the anti-viral action of interferon. Enhancement of cell-mediated tumor immunity was produced in mice exposed chronically to 100 ppm arsenic in the drinking

water. Humoral immunity of mice is suppressed by exposure to 0.5 to 10 ppm of arsenite in the drinking water. The suppression is present even at very low exposure levels (Squibb and Fowler, 1983).

A review by Willhite and Ferm (1984) states that prenatal death or malformations of the axial skeleton, neurocranium, viscerocranium, eyes, and genitourinary system are caused by arsenic. Sodium arsenite is more teratogenic than sodium arsenate, and arsenate more than methylarsonic acid or dimethylarsinic acid. Methylarsonic acid and dimethylarsinic acid must be given in very high doses to be teratogenic. Other organic arsenicals are also weakly or not teratogenic. Stage of gestation when exposed to arsenic is important. Sodium arsenite at five mg/kg of body weight given intraperitoneally on day nine or ten of gestation in hamsters causes resorption of all embryos. The same dose given day 11 or 12 results in decrease of fetal body weight but no gross abnormalities.

Squibb and Fowler (1983) have reviewed the literature on the carcinogenic effect of arsenic. Epidemiological studies suggest arsenic exposure increases the incidence of many types of cancer in humans. This association is not supported by experimental studies. In some studies arsenic appears to cause cancer and in others prevent it. The role of arsenic

as a cancer causing agent or enhancer of known carcinogens is yet to be defined.

SECTION I. EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON FEED INTAKE AND BODY WEIGHT OF DOGS

EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON FEED INTAKE AND BODY WEIGHT OF DOGS

(Effect of arsenite on dog feed intake)

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ABSTRACT

Thirty female beagle dogs, 7 to 8 months old, were assigned to 5 groups. Control, low dosage, medium dosage, high dosage, and pair fed groups were offered 0, 1, 2, 4, and 0 mg of sodium arsenite per kg of body weight per day (mg/kg/day) respectively in their feed. On day 59 the dosage was doubled for the rest of the experiment which ended on day 183. Feed consumption was measured daily and the body weight weekly. The pair fed group was offered the amount of feed that the high dosage group consumed. High dosage and pair fed groups were terminated in week 17 when the high dosage group had lost a cumulative average of 20 percent of their original body weight. Nominal dosages of 4 and 8 mg/kg/day caused significant decrease in feed consumption. Initial decreased feed consumption was followed by increased intake over time. Dogs offered 8 mg/kg/day did not fully compensate the lost feed intake. Nominal dosages of 4 and 8 mg/kg/day caused a significant decrease in body weight. The linear regression of pair fed group body weight over time was not significantly different than that of high dosage group. This study shows that dietary sodium arsenite causes a dose dependent decrease of feed consumption and body weight. Weight loss is caused by decreased feed consumption not by direct effect of the sodium arsenite.

INTRODUCTION

Arsenic is ubiquitous and present in rock, soil, water, and living organisms. Therefore it is impossible for man or animals to avoid exposure. In addition to natural sources, the frequent use of arsenicals as herbicides and insecticidal baits creates the potential for accidental exposure to higher concentrations of arsenic. Past experimentation in dogs lacks detailed evaluation of the concentration at which dietary arsenic causes effects and what the effects are (Calvery et al., 1938; Byron et al., 1967).

High dosage of arsenic results in dramatic acute clinical signs and lesions (Sullivan and Gruen, 1985; Osweiler et al., 1985; NAS, 1977). Information about chronic effects, primarily from human epidemiological studies, associate arsenic with degenerative, inflammatory, and neoplastic changes of the skin, respiratory system, liver, cardiovascular system, blood, lymphatic system, nervous system, and reproductive system (Pershagen, 1983). Long term studies are needed to determine neoplastic effects of arsenic. This study was designed to determine the maxium dosage that a dog could tolerate in a chronic study.

Numerous controlled subchronic and chronic inorganic arsenic studies have been done with rats and a lesser number with mice (Byron et al., 1967; Schroeder and Balassa, 1967;

Schroeder et al., 1968; Kiyono et al., 1974; Fowler et al., 1977; Fowler and Woods, 1979). Inorganic arsenic adversely effects the weight or weight gain of rats and mice. More controlled chronic studies in higher animals are needed, especially since rats metabolize arsenic differently than other species studied (Ducoff et al., 1948; Lanz et al., 1950; Klaassen, 1974).

In limited studies inorganic arsenic also adversely affected the weight of pigs and dogs (Byron et al., 1967; Morrison and Chavez, 1983). Dogs chronically fed 0.5 or 2.0 mg of arsenic per kg of body weight daily had no adverse effects (Calvery et al., 1938). The arsenic was in the form of finely powdered arsenic trioxide. In another study, dogs given feed containing 125 ppm of arsenic as sodium arsenate or sodium arsenite had significant weight loss (Byron et al., 1967). Weight loss was more severe in the group fed arsenite. Dogs exposed to 50 ppm of arsenic of either compound were not affected.

Feed or water is rejected by animals when it is contaminated by a high enough concentration of inorganic arsenic (Fowler and Woods, 1979; Morrison and Chavez, 1983). Therefore, feed rejection is probably the cause of the weight lost described above.

Sodium arsenite was used in this study. It is one of the most toxic forms of inorganic arsenic because it is water

soluble and trivalent (Squibb and Fowler, 1983).

The purpose of this study was to determine the dosage of dietary sodium arsenite that causes weight loss and whether the weight loss is a direct or indirect result of the arsenic.

MATERIALS AND METHODS

Thirty female beagle dogs, 7 to 8 month old, were randomly assigned to 5 groups. Control, low dosage, medium dosage, and high dosage groups were offered 0, 1, 2, or 4 mg of sodium arsenite ($NaAsO_2$) per kilogram of body weight in the feed daily. After 58 days, the dosage of all groups was doubled for the rest of the experiment. To account for substantial feed refusal in principals a pair fed group (inanition control) was fed the amount of feed, without sodium arsenite, that the high dosage group consumed on a percent body weight basis. Pairings were by group, not individual animals, and readjusted weekly to mimic intake of the high dosage group.

Each dog of the control, low dosage, medium dosage, and high dosage groups was offered daily 2.75 percent of its body weight of dry Purina High Pro Dog Chow (Ralston Purina Co., St. Louis, MO) for the first 2 weeks, then 3.00 percent for the rest of the experiment. Feed samples were quantitated for arsenic by hydride atomic absorption spectroscopy (303 Perkin-Elmer Atomic Absorption Spectrometer, Perkin-Elmer MHS-10 Hydride System, Perkin-Elmer Co., Norwark, Conn) according to methods previously described (Hyde et al., 1977). Feed contained less than 0.1 ppm arsenic. Stainless steel feed and water bowls were used. Dry feed was mixed

with an equal weight of water containing the appropriate dose of sodium arsenite dissolved in it. The feed was weighed and mixed with arsenic every morning. Feed and water were mixed thoroughly to insure even distribution of arsenic in feed. The amount of water added wet the feed evenly, and no excess water was left free of the feed. The mixture was offered to the dog for 6 to 8 hours. Each night the unconsumed feed was weighed and recorded. The dogs had free choice tap water at all times. Tap water contained less than 0.01 ppm of arsenic.

The dogs were housed individually in stainless steel cages (Shor-Line, Shroer Manufacturing Co., Kansas City, MO). Each unit consisted of 2 cages, one above the other. The assignment of dogs to cages was random except the bottom dog was always from the same group as the top dog. The dogs were observed at least 3 times a day, and the cages cleaned daily.

Body weights were determined and the amount of feed and arsenic adjusted weekly. To decrease the effect of variation of individual body weights, the weekly body weight of a dog was divided by the initial body weight of that dog. The fraction of the initial body weight (FIBW) of each animal was used in the analysis of data. Therefore, the FIBW of all dogs on week 1 was 1.

The experiment was divided into 3 phases (I, IIa and IIb) on the basis of time and groups for analysis of the data

generated (Table 1). Phase I involved all groups from week 1 through week 8. In phase I nominal dosages (intended dosage if dog consumed all feed offered to it) of sodium arsenite for control, low dosage, medium dosage, high dosage, and pair fed groups were 0, 1, 2, 4, and 0 mg per kg of body weight per day (mg/kg/day), respectively. Phase IIa consisted of all groups from weeks 9 through 17 at nominal dosages double that of phase I (0, 2, 4, 8, 0 mg/kg/day). At the end of phase IIa, high dosage and pair fed groups were terminated when the high dosage group had lost approximately 20 percent of their original body weight (Figure 13). Phase IIb consisted of control, low dosage, and medium dosage groups from week 9 to 26 at 0, 2, and 4 mg/kg/day, respectively. In all phases, the actual arsenic dosages were calculated daily by multiplying the percent of offered feed consumed by the appropriate nominal dosage. At the feeding rate of 3 percent of body weight per day, the phase I dosages were equivalent to 33.4, 66.7, and 133.4 ug of sodium arsenite per gm of dry feed for low, medium, and high dosage groups, respectively.

Pair fed group was started on the experiment 20 weeks after the other groups. Therefore, the data for a certain experimental week was on the average collected 20 weeks later, real time, than data for other groups. An average age difference of 20 weeks existed between pair fed group dogs

and dogs of other groups.

The experiment utilized a complete random design to compare groups with different treatments. Individual dogs were the experimental units. The time effect was a repeated measure and analyzed as a split plot factor. It was recognized that the week factor was a repeated measure and hence the significance levels of week and group by week interaction effects may have been exaggerated. However, we took account of this effect by using conservative degrees of freedom. Least significant differences were calculated from the experimental error of dogs within groups (Snedecor and Cochran, 1967). The data were analyzed using the SAS computer based system (SAS Institute Inc., Cary, NC).

RESULTS

Pair fed group feed consumption data were not analyzed with the other groups because it was not independent of high dosage group data. A separate analysis of high dosage and pair fed groups showed no statistical difference in the feed consumption.

High dosage group feed intake decreased significantly in week 1 relative to the week prior to arsenic exposure (Figure 1). High dosage group week 1 intake was statistically less than that of control and low dosage groups ($p<0.05$). By week 4, there was no significant difference among the groups. The other groups feed intake did not decrease relative to the control group. Linear regression of phase I feed consumption (Figure 2) demonstrated that the medium dosage group ($p<0.05$) and high dosage group ($p<0.01$) had significantly larger slopes than control and low dosage groups. The high dosage group slope was greater than the medium dosage group slope ($p<0.01$).

In phase IIa, weeks 9 through 17, the dosage was doubled for all groups. Both medium and high dosage groups had decreases in feed intake from week 8 to 9 (Figure 3). During week 9, medium dosage group feed consumption was not significantly less than the controls ($p<0.05$). High dosage group feed consumption was significantly less than control, low dosage ($p<0.01$) and medium dosage groups ($p<0.05$). By

week 17, consumption in medium and high dosage groups increased. However, high dosage group intake was still significantly less than control group ($p<0.05$). Linear regression analysis of phase IIa data (Figure 4) showed all three arsenic exposed groups had statistically different ($p<0.01$) feed intake slopes than controls. Medium and high dosage groups had a positive slope and low dosage group a negative slope relative to control group.

High dosage group was terminated on week 17. Control, low dosage, and medium dosage groups were continued through week 26 (phase IIb, Table 1). The medium dosage group had reduced feed intake in week 9 (not statistically significant), but feed intake increased and approached the level of controls and low dosage groups by week 26 (Figure 5). Linear regression slope of medium dosage group (Figure 6) was significantly greater ($p<0.01$) than slope for control and low dosage groups.

According to the experimental design, arsenic consumption was determined by the feed intake. The daily arsenic intake averaged over weeks was almost always significantly different among groups (largest $p <0.05$). The exceptions are between low and medium dosage groups in week 10 and between medium and high dosage groups in weeks 9, 10, 11, 12, 16 and 17. Average sodium arsenite consumption over

all of phase I was 0.0, 0.88, 1.80, 2.88, and 0.0 mg/kg/day for control, low dosage, medium dosage, high dosage, and pair fed groups, respectively. These overall averages are statistically different from each other ($p<0.01$). Arsenic consumption from week 1 to 8 was relatively constant for low and medium dosage groups. High dosage group sodium arsenite daily intake gradually increased through phase I. Linear regression for arsenic consumption in phase I (Figure 7) showed high dosage group consumption increased significantly ($p<0.01$).

In phase IIa, the overall average daily sodium arsenite consumption was 0.0, 1.66, 2.86, 3.74, and 0.0 mg/kg/day for control, low dosage, medium dosage, high dosage, and pair fed groups respectively. The overall averages are statistically different ($p<0.01$). Linear regression (Figure 8) showed significant increases in medium and high dosage groups arsenic intake from week 9 to 17 ($p<0.05$ and $p<0.01$ respectively). Low dosage group intake ($p<0.05$) decreased over the same period. In week 11, medium and high dosage groups consumed similar amounts of arsenite (2.77 and 2.83 mg/kg/day respectively) because of severe feed rejection by the high dosage group.

Control, low dosage, and medium dosage group average daily sodium arsenite consumption for phase IIb was 0.0, 1.59, and 3.00 mg/kg/day respectively. Average arsenic

intake amoung groups for phase IIb was statistically different ($p<0.01$). Arsenic intake decreased for the low dosage group and increased for the medium dosage group from weeks 9 to 26. Linear regression analysis (Figure 9) of the low dosage group decrease and medium dosage group increase was significant ($p<0.01$).

Sodium arsenite caused a dose dependent decrease in the FIBW. FIBW of the high dosage group decreased from week 0 to week 3 (Figure 10). From week 3 to week 8 the FIBW of the high dosage group leveled off and slightly increased. The linear regression of FIBW (Figure 11) over the first 8 weeks resulted in negative slopes for the high dosage and pair fed groups that were statistically similar and significantly less than those of other groups ($p<0.01$). Control, low dosage, and medium dosage groups had gradual weight gains over the same period that were not statistically different from each other.

FIBW of the pair fed group increased during week 1 to 1.050 then approximatley paralleled the weekly FIBW of high dosage group for the rest of phase I and IIa. This gave pair fed group a linear regression line higher than but almost parallel to that of high dosage group (Figures 11 and 12).

In phase IIa, control and low dosage groups continued to gain weight (Figure 13). Hence the linear regressions of

FIBW for these groups had positive slopes (Figure 12). Medium dosage group gradually lost weight and linear regression demonstrated a slightly negative slope. High dosage and pair fed groups lost weight faster than in phase I and had linear regression lines statistically parallel and with negative slopes. The regression slopes of medium dosage, high dosage, and pair fed groups were statistically less than those of control and low dosage groups ($p<0.01$). Slopes of high dosage and pair fed groups were significantly less than the slope of medium dosage group ($p<0.01$).

In phase IIb, medium dosage group gradually lost weight for the first half and then remained stable (Figure 14). This resulted in a linear regression line with a slightly negative slope (Figure 15). Both control and low dosage groups gradually gained weight over phase IIb, hence both had positively sloped linear regression lines. Low dosage group gained less than control group and had a significantly ($p<0.01$) smaller slope. Slope for medium dosage group is statistically less than those for either control or low dosage groups ($p<0.01$). There was significant, unexplained, weekly variation of FIBW ($p<0.01$).

DISCUSSION

Inorganic arsenic in the feed caused a dose dependent rejection of feed. Four mg/kg/day of sodium arsenite in the daily ration caused medium and high dosage groups to have decreases in feed intake the first week of phases IIa and I respectively. The high dosage group decrease was significant ($p<0.05$) and medium dosage group nearly significant ($p>0.05$, Figures 1 and 3). Eight mg/kg/day caused high dosage group to have a greater decrease ($p<0.01$) in intake than when it was first exposed to 4 mg/kg/day (Figure 3). All three decreases in feed intake were followed by a significant ($p<0.01$) increase of intake depicted by linear regression analysis (Figures 2 and 4).

Feed intake of control and low dosage groups were not significantly different in phases I and IIb (Figures 2 and 6). In phase IIa (Figure 4), the linear regression slopes of these groups was significantly different ($p<0.05$). When these data were reanalyzed with additional data in phase IIb, there was no statistical difference. This supports the conclusion that exposure to 1 or 2 mg/kg/day of sodium arsenite does not have a significant effect on feed intake. In phase I, medium dosage group (2 mg/kg BW /day) had a significant feed intake increase over time relative to controls (Figure 2). Medium dosage group week 8 feed

consumption was greater (statistically not significant) than that of the controls (Figure 1). This would suggest that arsenic at this concentration stimulates feed intake. However, the low dosage group exposed to 2 mg/kg/day in phase IIb did not exhibit the same change (Figure 6).

Dogs tolerated nominal dosages of 1 and 2 mg/kg/day of sodium arsenite (Figures 2 and 6). It appears that after a certain arsenic concentration is reached, the arsenic controls feed intake. When high dosage group exposure to arsenic was increased from 4 to 8 mg/kg/day, the absolute amount of arsenic consumed was not markedly increased for 6 weeks (Figures 7 and 8). This is because when arsenite concentrations were doubled for the high dosage group, the feed intake voluntarily reduced by half (Figure 3). Midway through phase IIa this trend reversed and the high dosage group started to gradually increase its intake.

The linear regression analysis of FIBW over time demonstrates a significant ($p<0.01$) decrease in body weight when dogs are exposed to 4 or 8 mg/kg/day of sodium arsenite (Figures 11, 12, and 15). Control and low dosage groups gained weight over the same period. Weight loss has been demonstrated previously in pigs, mice, rats, and dogs exposed to inorganic arsenic (Calvery et al., 1938; Byron et al., 1967; Fowler and Woods, 1979; Morrison and Chavez, 1983).

Regression line slopes for FIBW of high dosage and pair

fed groups were statistically the same. This demonstrates that decreased feed intake, not the direct effect of arsenic, caused the weight decrease. In Figure 11, the regression line for pair fed group is higher than high dosage group. This was caused by the unexplained increase of FIBW for pair fed group in week 1.

Hairless mice given 50 mg/liter of arsenic trioxide in the drinking water had an initial weight loss , but compensated over the following 15 weeks and achieved final weights similar to the controls (Bencko and Symon, 1969). Dogs in this study did not demonstrate this weight compensation. However, dogs offered 4 mg/kg/day had increasing feed intake after an initial decrease and if the study had been longer the decreased weights may have increased toward normal or control values.

It is not known how arsenic causes feed rejection. Following is a hypothesis formed from some of our observations.

The vomiting center of the medulla can be stimulated in 5 basic ways: by the 1) gastrointestinal tract, 2) heart and other viscera, 3) chemoreceptor trigger zones, 4) labyrinth and 5) supramedullary sites of the central nervous system (Barnes, 1984). In our laboratory, we have observed that high dosages of oral inorganic arsenic caused vomiting.

Vomiting was also produced by parenteral administration of sodium arsenite solution. The gastrointestinal tract and chemoreceptor trigger zone would be logical routes for arsenic stimulation of the vomiting center. In the gastrointestinal tract, local receptors are stimulated and send a nerve impulse via afferent nerves to the vomiting center in the brain stem. However, since intravenous administration of inorganic arsenic induces vomiting, the blood arsenic could be stimulating receptors of the chemoreceptor trigger zone which in turn stimulates the vomiting center. The chemoreceptor trigger zone is located in the area postrema of the medulla oblongata. It is stimulated by blood-borne chemical substances that cause vomiting. Known examples are digitalis, apomorphine, morphine and bacterial toxins (Davis, 1980; Borison et al., 1984).

We also subjectively observed taste aversion induced by feed contaminated with sodium arsenite. Dogs would eat a specific flavor of dog food once or twice when it contained large amounts of arsenite. Vomiting occurred shortly after ingestion. After one or two feedings, the dog would reject that type of dog food even if it did not contain arsenite in subsequent exposures. However, if feed type or flavor was changed the dog would eat it even if it contained arsenite. Taste aversion has been associated with the area postrema in

rats (Borison et al., 1984). Our observations of vomiting and taste aversion caused by arsenite are subjective and controled experiments should be done to verify them.

Nausea in humans is the conscious awareness of unusual activity in the centers associated with vomiting mediated by the central nervous pathway (Barnes, 1984). If dogs experience nausea induced by low levels of ingested arsenic this may be the reason that our high dose group rejected a large portion of their feed. This seems a plausible explanation that should be explored.

Another possible explanation of the lowered feed intake is that arsenic stimulated the satiety center of the brain stem and the dogs were not hungry (Sullivan and Gruen, 1985). High dosage dogs (8 mg/kg/day) were continually at the feed bowl, worrying, but not completely ingesting the daily feed offering. This suggests the dogs were hungry and the satiety center of the brain was not stimulated.

Without specifically knowing the mechanism for the arsenic induced feed rejection, we suggest that rejection was due to blood borne arsenic stimulation of the chemorecptor trigger zone which excited the vomiting center and produced nausea without vomiting. Arsenic treated dogs in our study did not have more vomiting activity than controls.

Gradual feed intake increasing after the initial

rejection of feed containing 4 or 8 mg/kg/day of sodium arsenite suggests that adaptation was occurring (Figures 2, 4, and 6). If nausea was the cause of feed rejection, it is possible the dogs got so hungry that appetitive drive became the stronger behavioral stimulus. Feed aversion induced by illness in rats is weakened by increased hunger (Peters and Reich, 1973).

Adaptation to arsenic does occur and has been shown experimentally to increase the arsenic LD_{50} in mice chronically exposed to arsenic trioxide (Bencko and Symon, 1969). This might be accomplished by decreased absorption or increased excretion of ingested arsenic. If either of these occurred, presumably blood arsenic levels would remain below a level that stimulates the chemoreceptor trigger zone.

It has been shown in rats exposed to oral inorganic trivalent arsenic that there is an increase of glutathione in the intestinal mucosa. This could block systemic arsenic absorption by chelation (Pisciotto and Graziano, 1980).

Adaptation due to increased excretion is possible. The largest portion of the body burden of inorganic arsenic is eliminated by the urine (Vahter, 1983). Chronic exposure of mice to low concentrations of inorganic arsenic in the water decreased organ arsenic retention after subsequent subcutaneous injection of arsenic (Bencko et al., 1973). Urinary excretion is more efficient when arsenic is

methylated (Marafante and Vahter, 1984; Marafante et al., 1985). Fibroblasts in culture pretreated with low doses of trivalent arsenic increased biotransformation of inorganic arsenic to methylated forms (Fischer et al., 1985). This suggests that mammals adapt to arsenic by increased methylation and therefore increased urinary excretion.

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TABLE 1

Nominal Dosages[a] in Study of Dogs

Offered Sodium Arsenite Contaminated Feed

	Group[b]				
	Control	Low Dosage	Medium Dosage	High Dosage	Pair Fed[c]
Phase I (weeks 1-8)	0	1	2	4	0
Phase IIa (weeks 9-17)	0	2	4	8	0
Phase IIb (weeks 9-26)	0	2	4		

[a]Mg of sodium arsenite offered daily per kg of body weight.

[b]Six dogs in each group.

[c]Pair fed amount of feed equal to high dosage group feed intake (without arsenic).

Fig. 1. Average daily feed consumption as percent of body weight for selected weeks in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair fed groups respectively. High dose and pair fed group week 1 consumption was significantly lower than that of controls ($p<0.05$).

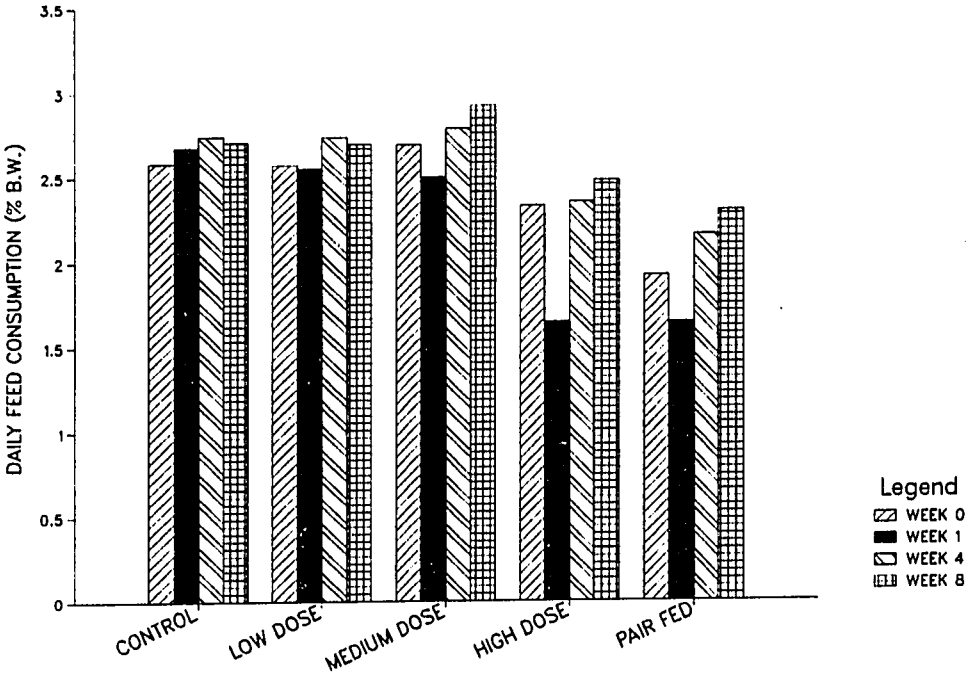

DAILY FEED CONSUMPTION (% B.W.)
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED
Legend
WEEK 0
WEEK 1
WEEK 4
WEEK 8

Fig. 2. Linear regression of daily feed consumption as percent body weight over time in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. Medium and high dose group slopes were significantly higher than that of controls ($p<0.05$ and $p<0.01$, respectively).

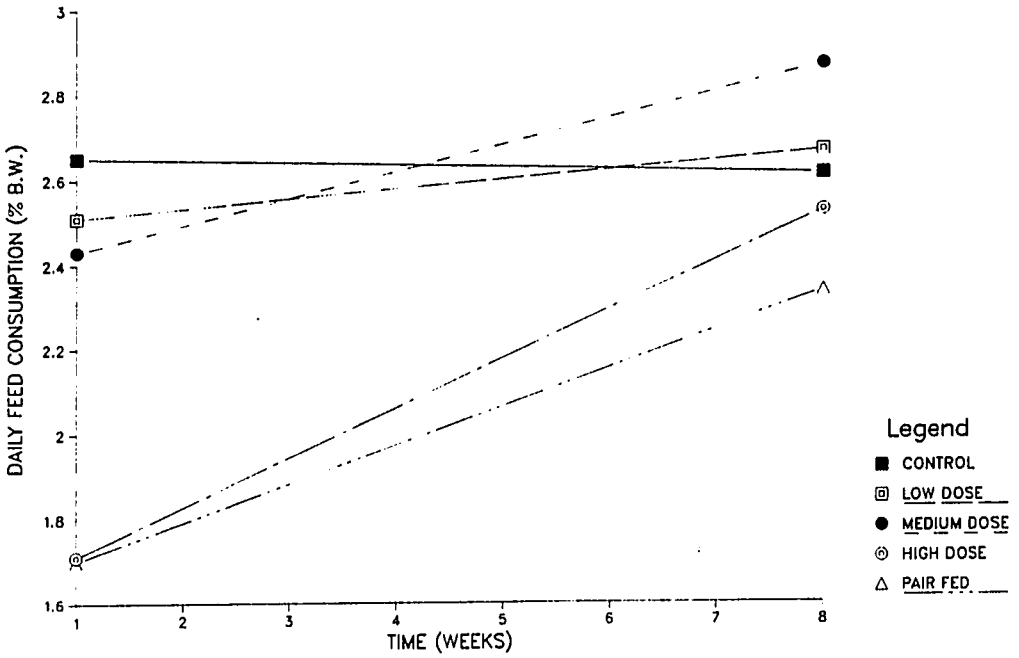

DAILY FEED CONSUMPTION (% B.W.)
3
2.8
2.6
2.4
2.2
2
1.8
1.6
1
2
3
4
5
6
7
8
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED

Fig. 3. Average daily feed consumption as percent of body weight for the last week of phase I and selected weeks in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8,and 0 mg/kg/day for control, low, medium, high, and pair fed groups respectively. Feed consumption of the high and pair fed groups were significantly lower than the controls in weeks 9, 13 ($p<0.01$), and 17 ($p<0.05$).

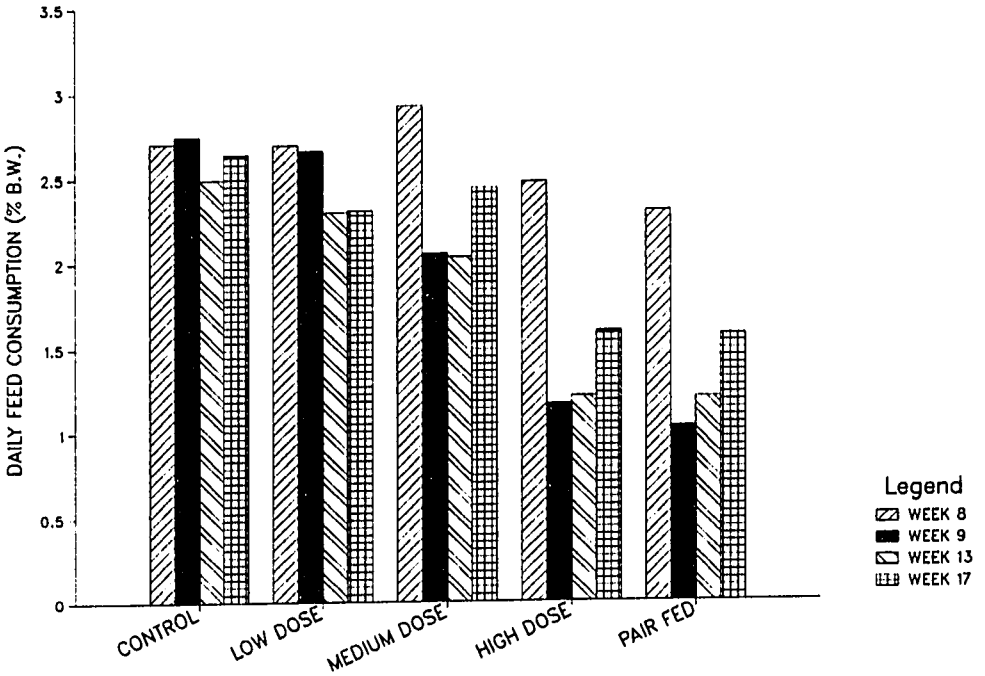

DAILY FEED CONSUMPTION (% B.W.)
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED
Legend
WEEK 8
WEEK 9
WEEK 13
WEEK 17

Fig. 4. Linear regression of daily feed consumption as percent body weight over time in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. Slopes for medium dose, high dose, and pair fed groups are significantly higher ($p<0.01$) and the slope of the low dose group lower ($p<0.05$) than that of controls.

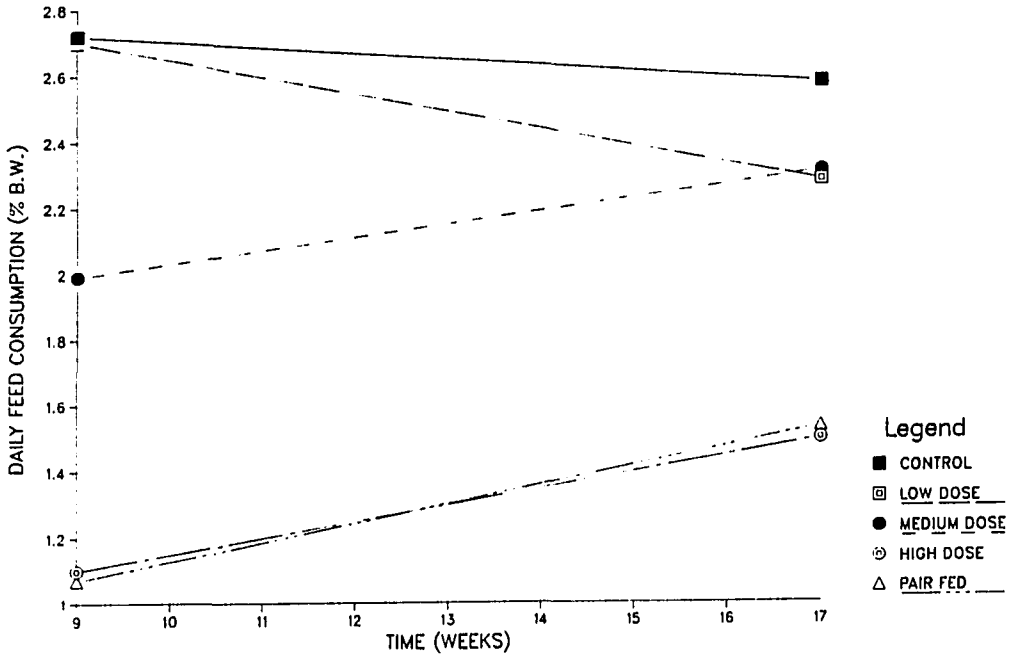

DAILY FEED CONSUMPTION (% B.W.)
2.8
2.6
2.4
2.2
2
1.8
1.6
1.4
1.2
1
9
10
11
12
13
14
15
16
17
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED

Fig. 5. Average daily feed consumption as percent of body weight for selected weeks in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups respectively. No weekly group averages are significantly different.

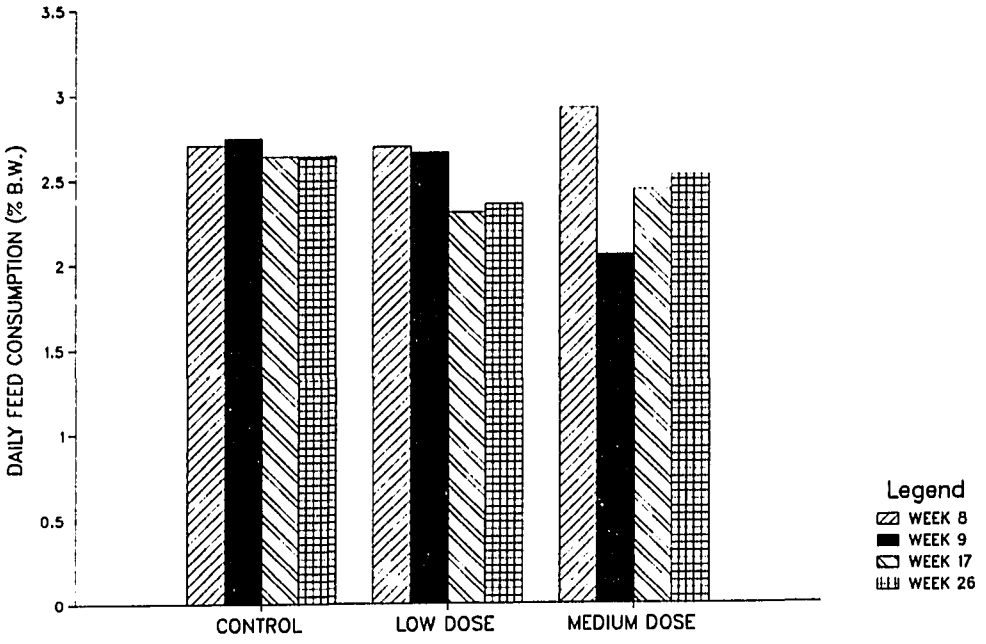

DAILY FEED CONSUMPTION (% B.W.)
3.5
3
2.5
2
1.5
1
0.5
0
CONTROL
LOW DOSE
MEDIUM DOSE
Legend
WEEK 8
WEEK 9
WEEK 17
WEEK 26

Fig. 6. Linear regression of daily feed consumption as percent body weight over time in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups respectively. Medium dose group slope is significantly higher than slopes for low dose and control groups ($p<0.01$).

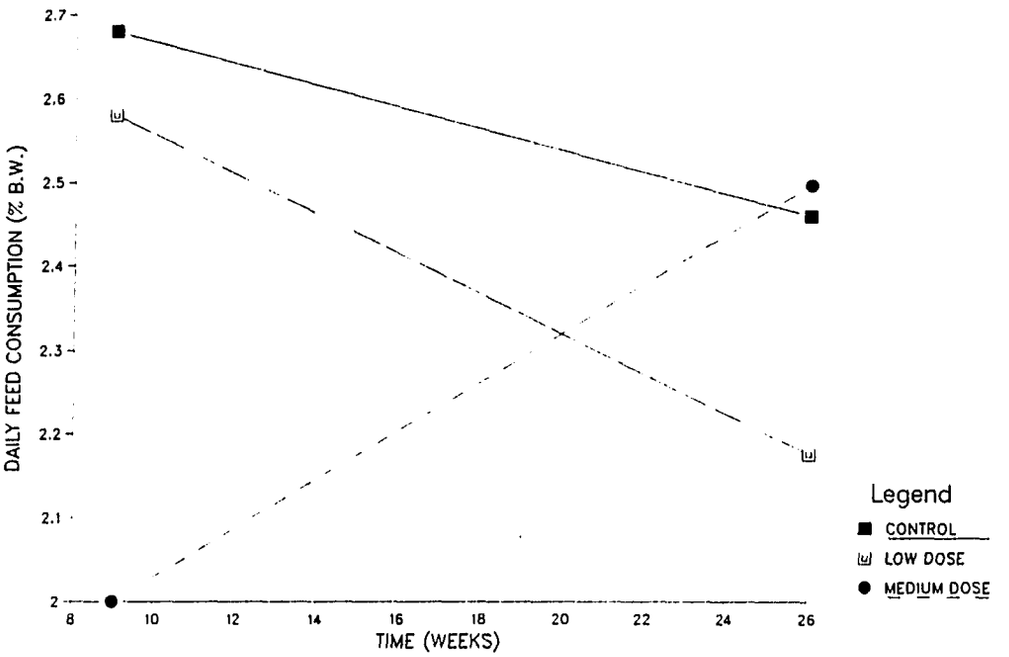

DAILY FEED CONSUMPTION (% B.W.)
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
8
10
12
14
16
18
20
22
24
26
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE

Fig. 7. Linear regression of actual daily sodium arsenite consumption as mg per kg of body weight over time in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, and 4 mg/kg/day for control, low, medium, and high dose groups, respectively. High dose group slope is significantly higher than slopes for low, medium, and control groups ($p<0.01$).

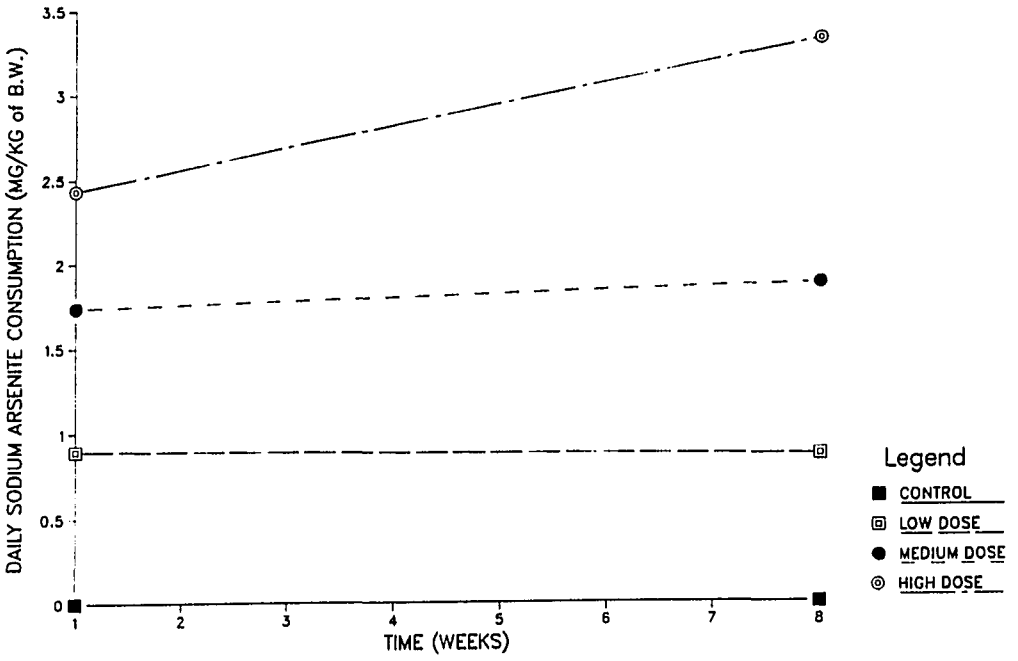

DAILY SODIUM ARSENITE CONSUMPTION (MG/KG of B.W.)
3.5
3
2.5
2
1.5
1
0.5
0
1
2
3
4
5
6
7
8
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE

Fig. 8. Linear regression of actual daily sodium arsenite consumption as mg per kg of body weight over time in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, and 8 mg/kg/day for control, low, medium, and high dose groups, respectively. High and medium dose group slopes are significantly higher ($p<0.01$ and $p<0.05$ respectively) and low dose group slope significantly lower ($p<0.05$) than that of control group.

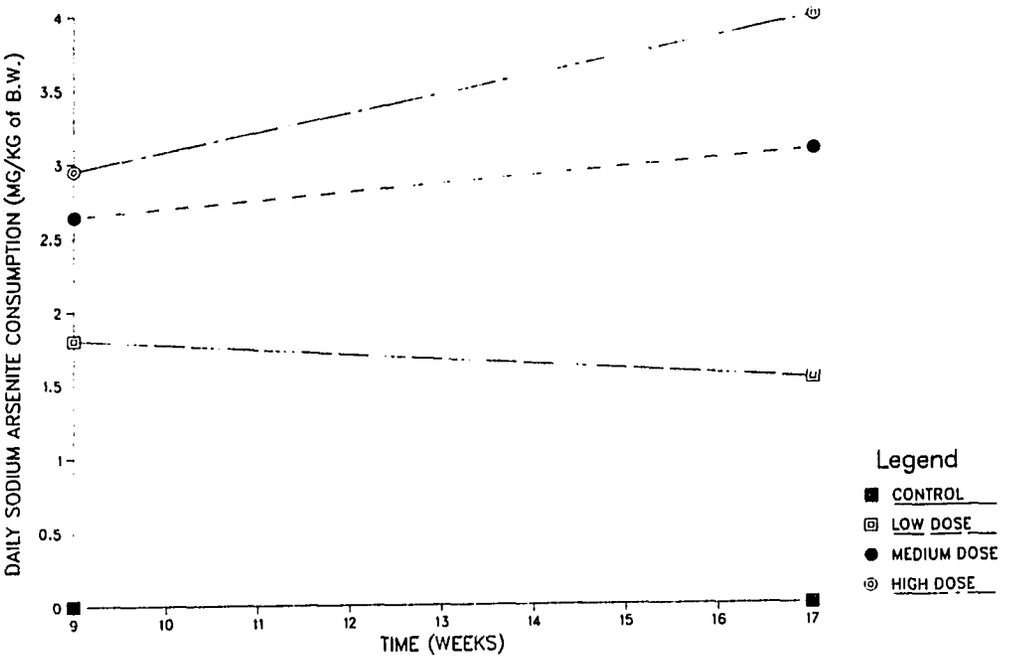

DAILY SODIUM ARSENITE CONSUMPTION (MG/KG of B.W.)
4
3.5
3
2.5
2
1.5
1
0.5
0
9
10
11
12
13
14
15
16
17
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE

Fig. 9. Linear regression of actual daily sodium arsenite consumption as mg per kg of body weight over time in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups, respectively. Medium dose group slope is significantly higher and low dose group slope significantly lower than that of control group ($p<0.1$).

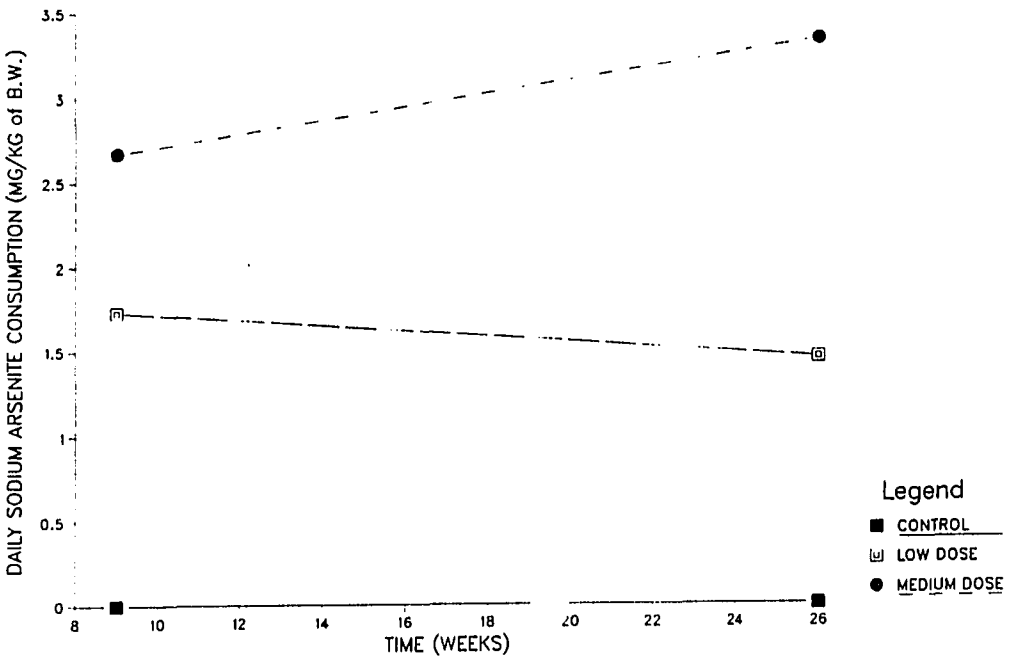

DAILY SODIUM ARSENITE CONSUMPTION (MG/KG of B.W.)
3.5
3
2.5
2
1.5
1
0.5
0
8
10
12
14
16
18
20
22
24
26
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE

Fig. 10. Average fraction of initial body weight for selected weeks in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. No significant differences between the weekly averages were observed ($p<0.05$).

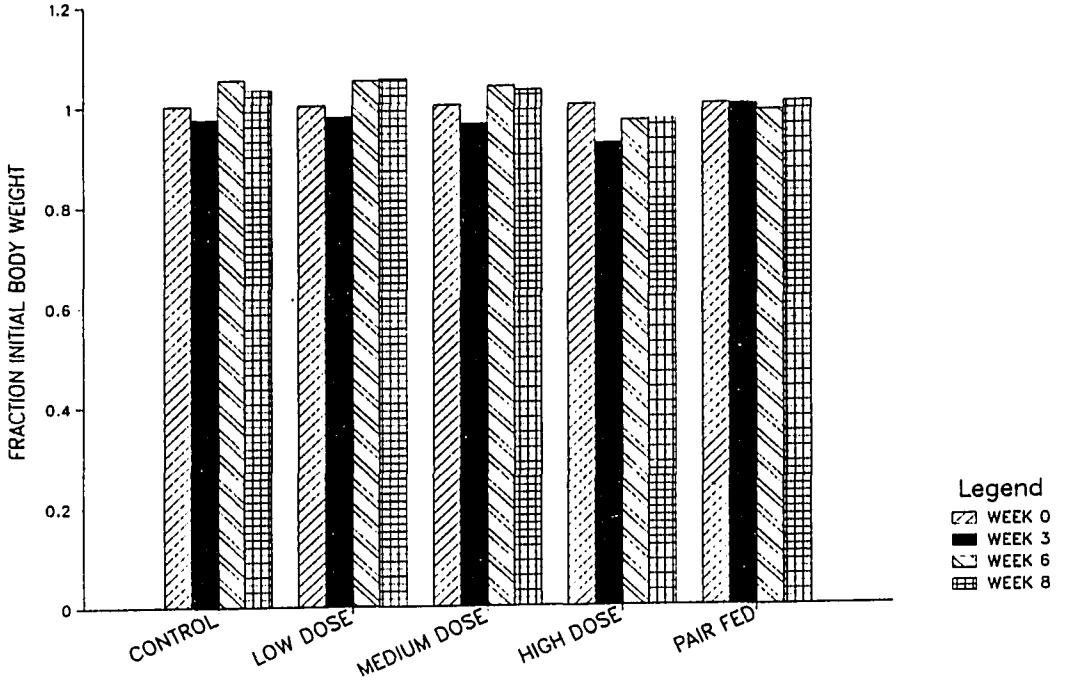

FRACTION INITIAL BODY WEIGHT
1.2
1
0.8
0.6
0.4
0.2
0
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED
Legend
WEEK 0
WEEK 3
WEEK 6
WEEK 8

Fig. 11. Linear regression of the fraction of initial body weight over time in phase I. Nominal dosages of sodium arsenite were 0, 1, 2, 4, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. High and pair fed group slopes were statistically similar and significantly lower than that of the other groups ($p<0.01$).

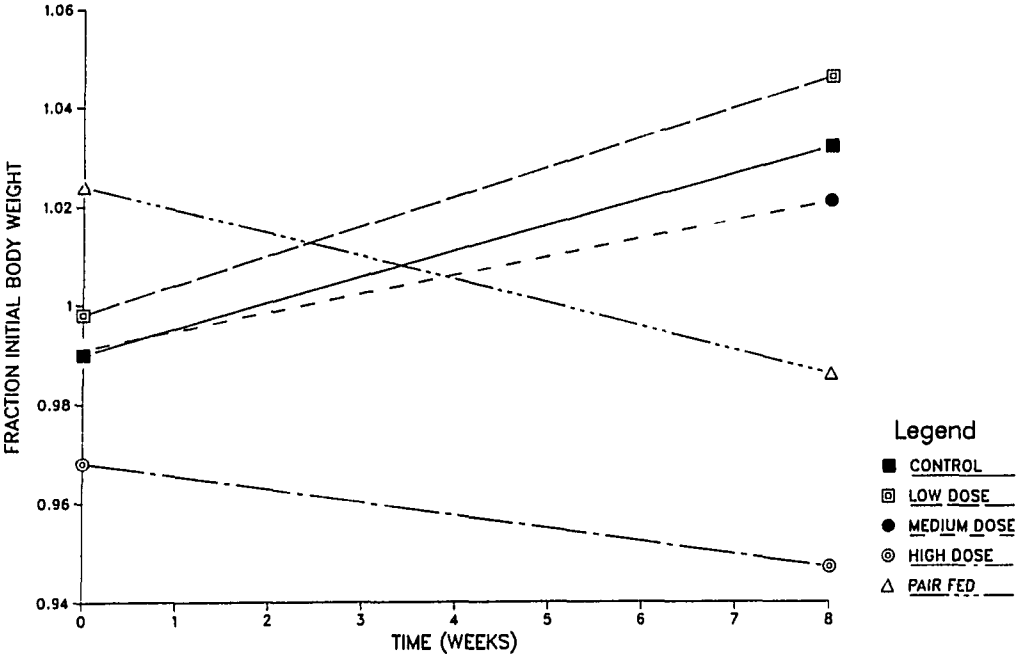

1.06
1.04
1.02
1
0.98
0.96
0.94
FRACTION INITIAL BODY WEIGHT
0
1
2
3
4
5
6
7
8
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED

Fig. 12. Linear regression of the fraction of initial body weight over time in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. High and pair fed group slopes were statistically similar and significantly lower than that of other groups ($p<0.01$). Medium dose group slope was significantly lower than slopes of low dose and control groups ($p<0.01$).

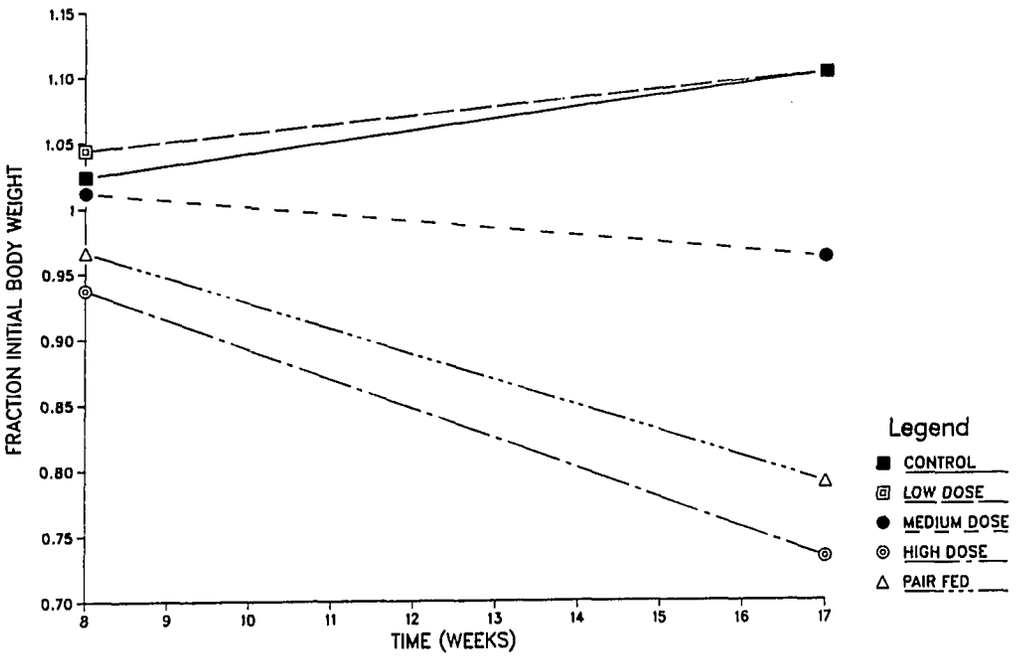

FRACTION INITIAL BODY WEIGHT
1.15
1.10
1.05
1
0.95
0.90
0.85
0.80
0.75
0.70
8
9
10
11
12
13
14
15
16
17
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED

Fig. 13. Average fraction of initial body weight for week 8 and selected weeks in phase IIa. Nominal dosages of sodium arsenite were 0, 2, 4, 8, and 0 mg/kg/day for control, low, medium, high, and pair fed groups, respectively. Averages for high dose group during weeks 14 and 17 are significantly lower than those of the controls ($p<0.05$).

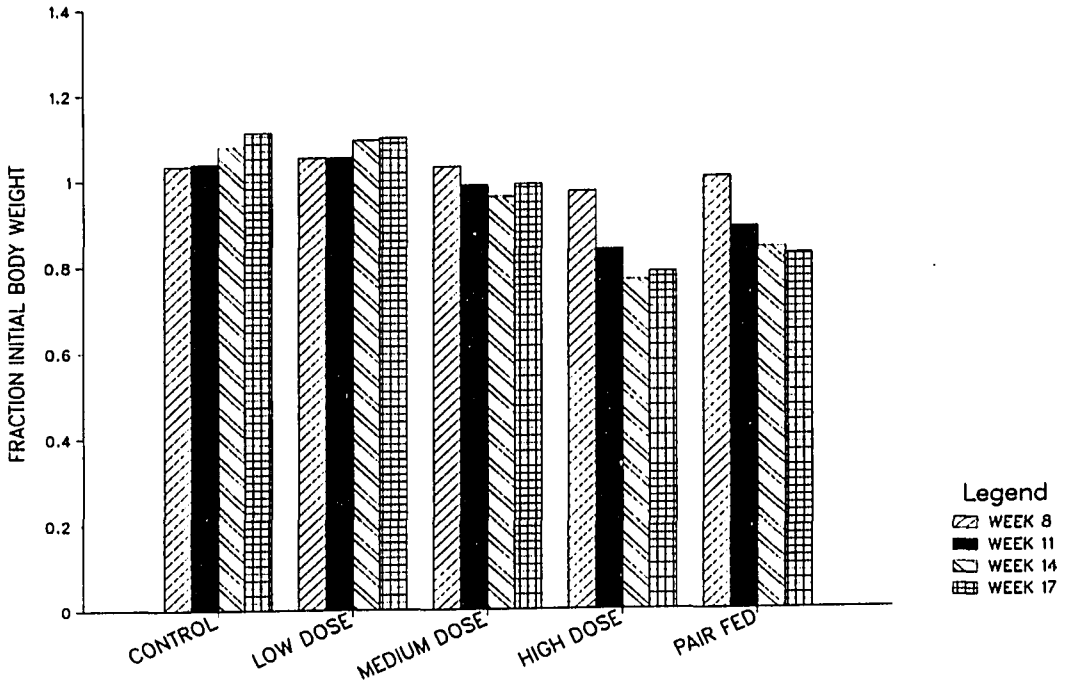

FRACTION INITIAL BODY WEIGHT
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CONTROL
LOW DOSE
MEDIUM DOSE
HIGH DOSE
PAIR FED
Legend
WEEK 8
WEEK 11
WEEK 14
WEEK 17

Fig. 14. Average fraction of initial body weight for week 8 and selected weeks in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups, respectively. No significant differences between the weekly averages are observed ($p<0.05$).

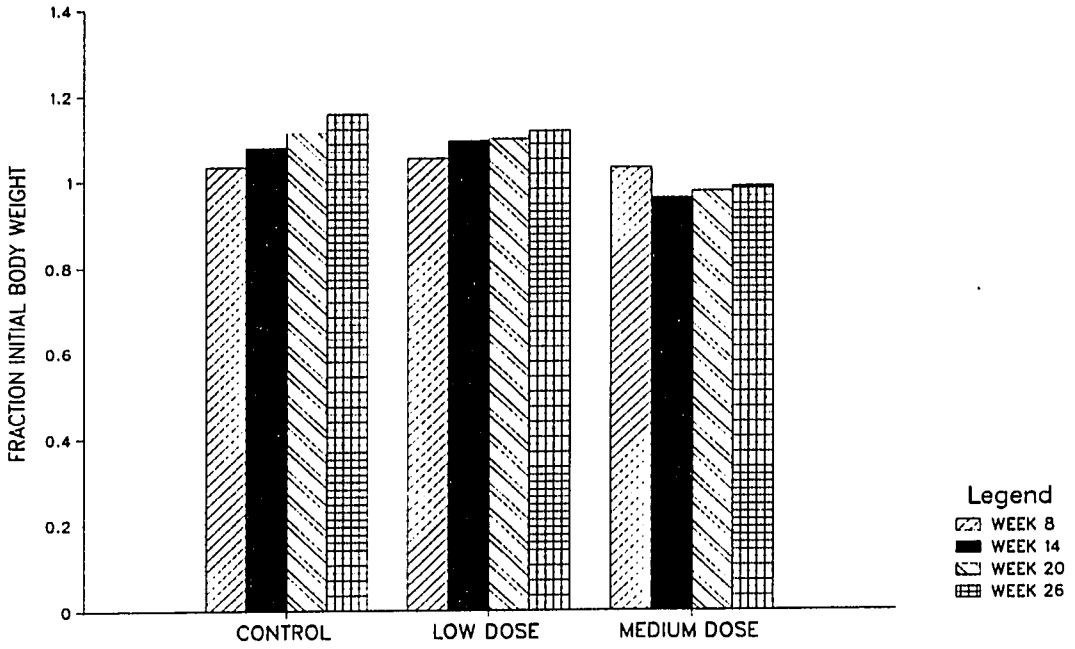
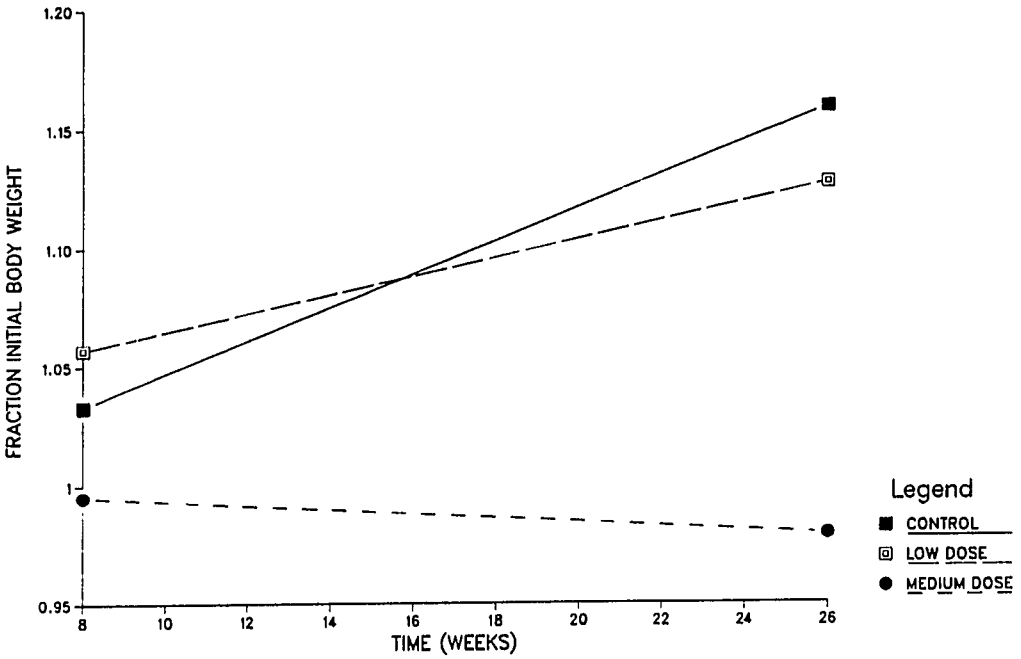

FRACTION INITIAL BODY WEIGHT
1.4
1.2
1
0.8
0.6
0.4
0.2
0
CONTROL
LOW DOSE
MEDIUM DOSE
Legend
WEEK 8
WEEK 14
WEEK 20
WEEK 26

Fig. 15. Linear regression of the fraction of initial body weight over time in phase IIb. Nominal dosages of sodium arsenite were 0, 2, and 4 mg/kg/day for control, low, and medium dose groups, respectively. The slopes of the low dose group and medium dose group are significantly lower than that of controls ($p<0.01$). Slope of medium dose group is significantly lower than that of low dose group ($p<0.01$).


1.20
1.15
1.10
1.05
1
0.95
FRACTION INITIAL BODY WEIGHT
8
10
12
14
16
18
20
22
24
26
TIME (WEEKS)
Legend
CONTROL
LOW DOSE
MEDIUM DOSE

SECTION II. EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON BLOOD, SERUM, AND URINE OF DOGS

EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON BLOOD, SERUM, AND URINE OF DOGS (Clinical pathology of As exposed dogs)

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ABSTRACT

Thirty female beagle dogs, 7 to 8 months old, were assigned to 5 groups. Control, low dosage, medium dosage, high dosage, and pair fed groups were offered 0, 1, 2, 4, and 0 mg of sodium arsenite per kg of body weight per day (mg/kg/day) respectively in their feed. On day 59 the dosage was doubled for the rest of the experiment which ended on day 183. The pair fed group was offered the amount of feed that the high dosage group consumed. High dosage and pair fed groups were terminated in week 17 when the high dosage group had lost a cumulative average of 20 percent of their original body weight. Serum liver leakage enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated in treated groups relative to controls. Serum AST was elevated in dogs exposed to 4 and 8 mg/kg/day of sodium arsenite. Serum ALT was elevated in dogs exposed to 2, 4, and 8 mg/kg/day. ALT and AST elevations relative to controls were persistent and low grade. Only ALT in the high dosage group was elevated above what is considered normal. Elevated serum ALT and AST indicates sodium arsenite causes altered hepatocyte plasma membrane permeability.

INTRODUCTION

Arsenic is ubiquitous in nature and local concentration increases, caused by man, are common. Therefore, it is impossible to avoid arsenic exposure at some level. Past experimentation in dogs lacks detailed evaluation of the concentration at which dietary arsenic causes effects and what the effects are (Calvery et al., 1938; Byron et al., 1967). Human epidemological studies have associated arsenic with a multitude of disorders involving almost every organ system of the body (Pershagen, 1983). Lesions range from hyperkeratosis of the skin to neoplasia of the liver. Controlled experimental studies have not as yet proven all the associations made by epidemological studies. Long term studies are needed to determine neoplastic effects of arsenic. This study was designed to determine the maxium dosage a dog could tolerate in a long term study without serious acute of chronic toxicosis.

It is well established that inorganic arsenicals affect the function of a large number of enzymes within many metabolic systems. Replacement of PO_4 with AsO_4 in important biochemical compounds such as ATP has also been documented (Squibb and Fowler, 1983). Therefore it may be assumed all organ systems in the body could be affected by arsenic.

In this study we monitored many organ systems repeatedly

by the use of a wide variety of clinical pathological methods. This was done to determine which organ systems are affected by dietary inorganic arsenic.

MATERIALS AND METHODS

Thirty female, 7 to 8 month old, beagle dogs were randomly assigned to five groups. Control, low dosage, medium dosage, and high dosage groups were offered 0, 1, 2, or 4 mg respectively of sodium arsenite ($NaAsO_2$) per kilogram of body weight in the feed daily (mg/kg/day). After 58 days the dosage of all groups was doubled for the rest of the experiment. To account for substantial feed refusal in principals a pair fed group (inanition control) was fed an amount of feed, without $NaAsO_2$, that the high dosage group consumed on a percent body weight basis. Pairings were by group, not individual animals, and readjusted weekly to mimic intake of the high dosage group.

Each dog of control, low dosage, medium dosage, and high dosage groups was offered daily 2.75 percent of their body weight of dry Purina High Pro Dog Chow (Ralston Purina Co., St. Louis, MO) for the first two weeks, then 3.00 percent for the rest of the experiment. Dry feed was mixed with an equal weight of water with the appropriate dose of sodium arsenite dissolved in it. The feed was weighed out and mixed with arsenic every morning. Feed and water were mixed thoroughly to insure even distribution of arsenic in feed. The amount of water added wet the feed evenly, and no excess water was left free of the feed. The mixture was offered to each dog

for 6 to 8 hours. Every night the unconsumed feed was weighed and recorded. Body weights were measured and the amount of feed and arsenic adjusted weekly. The dogs had free choice tap water at all times.

The dogs were housed individually in Shore-Line stainless steel cages (Shroer Manufacturing Co., Kansas City, MO). Each unit consisted of two cages, one on the top of the other. The assignment of dogs to cages was random except the bottom dog was always from the same group as the top dog. The dogs were observed at least three times a day, and the cages cleaned daily.

The experiment was divided into three phases (I, IIa and IIb) on the basis of time and groups for the purpose of proper analysis of the data generated. Phase I involved all groups from week 1 through week 8. In phase I nominal dosages (intended dosage if dog consumed all feed offered to it) of $NaAsO_2$ for control, low dosage, medium dosage, high dosage, and pair fed groups were 0, 1, 2, 4, and 0 mg/kg/day respectively. Phase IIa consisted of all groups from weeks 9 through 17 at nominal dosages double that of phase I (0, 2, 4, 8, 0 mg/kg/day). At the end of phase IIa, high dosage and pair fed groups were terminated when the high dosage group had lost approximately 20 percent of their original body weight. Phase IIb consisted of control, low dosage, and medium dosage groups from week 9 to 26 at 0, 2, and 4

mg/kg/day, respectively.

Pair fed dogs were started on the experiment 20 weeks after the other groups. Therefore, the data collected for any experimental week from the pair fed group were on the average collected 20 weeks later, real time, than data from control, low dosage, medium dosage, and high dosage groups. An average age difference of 20 weeks existed between pair fed dogs and dogs of the other groups.

Periodic blood, serum, and urine samples were taken from all dogs and analyzed for a wide range of parameters (Table 1). Due to the early termination of 2 dogs, of the high dosage group, they were sampled on week 15. Because of extenuating circumstances the pair fed group was not sampled on the exact trial day as the other groups. Urine was collected by transabdominal cystocentesis and every attempt was not successful due to empty bladders. At times it was 2 days before an attempt was successful so samples from different dogs were secured within a period of 2 to 3 days. Serum samples were taken at the same time urine samples were secured.

The following determinations were made on blood: packed cell volume (PCV) (Autocrit Ultra 3, Clay Adams, Becton Dickinson and Co., Parsippany NJ), total (WBC) (ZBI Coulter Counter, Coulter Electronics, Inc., Hialeah FL) and

differential (DIFF) white blood cell count (Ames Hemotech Slide Stainer, Ames Division, Miles Laboratories, Inc., Elkhart, IN), hemaglobin (HGB) (Coulter Hemoglobinometer, Coulter Electronics, Inc., Hialeah FL), total plasma protein (TPP), and fibrinogen (FIB) (TPP and FIB were measured on a standard T-S Meter, American Optical Company, Buffalo, NY). Difference between refractometer readings of heated (56^{o} C for 3 minutes) and unheated plasma was calculated as FIB content. In addition partial thromboplastin time (PTT) and prothrombin time (PT) were measured in control and high dosage groups after 1, 6, and 12 weeks on trial (Hyland Clotek Fibrometer, Hyland Diagnostics Division, Cooper Diagnostics, Malvern PA).

The serum was analyzed for urea nitrogen (BUN) (S.V.R.^{TM} BUN Test Kit, Calbiochem-Behring, La Jolla, CA), total bilirubin (TB), direct bilirubin (DB) (Serosonic Bilirubin Test Kit, Mallinckrodt Inc. St Louis, MO), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) (S.V.R.^{TM} AST, ALT, and ALP Test Kits, Calbiochem-Behring, La Jolla, CA) and albumin (ALB) (BCG reaction, Pierce reagents, Pierce Chemical Company, Rockford, IL). Additional blood was collected in sodium fluoride oxalate for evaluation of plasma glucose (GLU) (S.V.R.^{TM} GLU Test Kit, Calbiochem-Behring, La Jolla, CA) All photometric tests were run on a chemistry auto-analyzer

(Rotochem IIa: American Instrument Company, Silver Spring, MD)

Urine was checked for glucose, blood, ketones (acetoacetic acid), bilirubin, urobilinogen, protein, and pH (Multistix, Ames Division, Miles Laboratories, Inc., Elkhart, IN). Urine ALP was evaluated with method noted above. Urine ALP to urine creatinine ratios were calculated to eliminate the effect of urine concentration from the data analysis. The specific gravity of every urine sample was measured with a standard T-S Meter (American Optical Company, Buffalo, NY). Urine osmolality was also measured (Osmette A Osmometer, Precision Systems, Natick MA).

Concentrations of Na, K, Cl, P, and creatinine (CRT) were determined in the urine and serum of every animal for every test period. Serum Ca determinations were also done. Na and K were measured with flame photometry (343 Flame Photometer: Instrumentation Laboratory, Inc., Lexington, MA) and Cl by AgCl precipitation (Corning Model 920M Chloride Meter: Corning Scientific Instruments, Medfield, MA). Ca and P determinations were made on a chemistry auto-analyzer (Rotochem IIa: American Instrument Company, Silver Spring, MD) using the Worthington Inorganic Phosphorus Reagent Set (Worthington Diagnostic Systems, Inc., Freehold, NJ) and the cresolphthalon complexone-diethylamine reaction with Ca.

GilChem reagents (Ciba Corning Diagnostics, Oberlin OH) were used in creatinine determinations to produce a picrate-creatinine color producing complex which was measured by a spectrophotometer (Gilford 103 Spectrophotometer, Ciba Corning Diagnostics, Oberlin OH). This method removes pseudocreatinine from the determination.

Simultaneous serum and urine values were used to calculate the percent clearance ratios of Na, K, Cl, and P. Percent clearance ratio is defined by the following formula:

$$PCR \text{ of } X = [\ (X_{ur}/X_{sr})\ /\ (CR_{ur}/Cr_{sr})\ ]\ *\ 100$$

where X_{ur} is the concentration of substance X in the urine, X_{sr} is the concentration of substance X in the serum, CR_{ur} is the concentration of creatinine in the urine, and CR_{sr} is the concentration of creatinine in the serum. Dogs secrete a small amount of creatinine from the renal proximal tubules. Creatinine secretion is greater in males and its clearance overestimates the glomerular filtration rate (Robinson et al., 1974). For this reason females were used.

The experiment was of complete random design used to compare groups of different treatments. Individual dogs were the experimental units. Least significant differences (LSD) were calculated from the experimental error of dogs within groups (Snedecor and Cochran, 1967). The data were analyzed using the computer based Statistical Analysis Systems (SAS Institute Inc., Cary, North Carolina).

The blood, serum, and urine values of Phases I, IIa, and IIb were analyzed separately. Values for phases I and IIa were analyzed via the dog means for that phase without consideration of time since there were only 2 samples taken per dog in each phase. This was not enough for a valid analysis of the time factor. Phase IIb data were analyzed with consideration of the time by group interaction. In analysis of phase IIb it was recognized that the time factor was a repeated measure and hence the significant levels of time by group effects may have been exaggerated. However, we took account of this effect by using conservative degrees of freedom when determining the probability of greater F values.

RESULTS

Dietary arsenite caused a statistically significant dosage dependent decrease in feed consumption and body weight (Neiger and Osweiler, 1987). Normal ranges used in this paper were defined by Duncan and Prasse (1986).

All group mean blood values were within the normal range. Blood values other than FIB of treated groups were not significantly different from controls. FIB was significantly elevated relative to controls in high dosage and pair fed groups in phase IIa (Table 2). Pair fed group elevation was significantly higher than that of high dosage group.

Mean PT and PTT combined values for control and high dosage dogs were 8.6 and 16.5 seconds respectively. There was no statistically significant difference between these groups ($p>0.05$).

All group mean serum values except ALT were within the normal range. No differences in serum creatinine, glucose, total bilirubin, direct bilirubin, and alkaline phosphatase were present among groups. Group differences were present in ALB, BUN, AST, and ALT levels. Phase IIa BUN levels of pair fed animals were depressed relative to controls ($p=0.01$). Serum ALB was elevated in medium dosage, high dosage, and pair fed groups in phase I ($p=0.03$). In phase IIa, high

dosage and pair fed groups had depressed serum ALB ($p=0.03$). In all phases AST and ALT were elevated in one or more treated groups (Table 3). Serum AST values were significantly elevated in high dosage dogs in phases I and IIa ($p<0.05$) and medium dosage dogs in phases IIa and IIb ($p<0.01$). Serum ALT values were significantly elevated in low dosage dogs in phase IIb ($p<0.05$), medium dosage dogs in phase IIb ($p<0.01$), and high dosage dogs in phase I and IIa ($p<0.01$). Pretreatment mean values for ALT and AST were 24 and 19 IU/L respectively. There was no statistically significant difference between pretreatment values of control and treated groups.

Urine blood, ketones, urobilinogen, protein, pH, specific gravity, and osmolality were within the normal range and no significant differences were present among groups. Urine bilirubin for the pair fed group was significantly elevated in phase I ($p=0.01$). In this period each dog was sampled twice (Table 1). Three of 6 pair fed dogs in this period had 1 sample which tested +1 or +2 and the other tested 0. All other dogs of all groups tested 0 on both samples.

Urine glucose was elevated in pair fed dogs during phase IIa. As was the case for urine bilirubin in phase I, 3 of the 6 pair fed dogs had 1 of 2 urine samples test 0.1g/dl and the other 0. All other dogs of other groups tested 0 in both

samples.

Urine ALP of the low dosage group was significantly elevated relative to controls in phase IIa and IIb ($p=0.01$ and $p<0.05$ respectively). No other group in any phase varied significantly from controls. Analysis of urine ALP expressed as raw data or urine ALP / urine CRT showed that the low dosage group deviated from controls. Mean urine ALP to CRT ratios for control and low dosage groups for phase IIa were 1.2 and 2 IU/g and for phase IIb were 1.1 and 1.6 IU/g respectively.

No significant difference in serum Cl was present among groups. Serum Na for low and medium dosage groups in phase I was increased significantly over control group ($p<0.05$). Phase I serum Ca was increased in the medium dosage group ($p<0.05$) and decreased in pair fed group ($p<0.01$) relative to controls. Phase IIa serum Ca of high dosage and pair fed dogs was significantly lower than that of control dogs ($p<0.01$ and $p<0.05$ respectively). Mean serum Ca for medium dosage was also lower than controls but not significantly. In phase IIb, the medium dosage group had lower serum Ca than the control group ($p<0.01$). Serum P values of pair fed dogs in phases I and IIa were lower than those of control dogs ($p<0.01$). Low dosage and medium dosage groups in phase IIb had higher serum K than the controls ($p<0.01$). Mean serum K

values were 4.2, 4.5, and 4.5 for control, low dosage, and medium dosage dogs respectively. The LSD was 0.29 ($p=0.01$).

PCRs for Na, K, P, and Cl were not significantly different among groups in any of the 3 phases.

The phase IIb analysis of time by group interaction showed no significant treatment effect in any of the parameters measured.

DISCUSSION

FIB in phase IIa was elevated in both high dosage and pair fed groups. Therefore arsenic can be ruled out as the cause. This increase was not above what is considered high normal. A major common factor in these groups was limited feed intake. It is not apparent how reduced feed intake could cause the increase. Normal TPP values for the same time period eliminates dehydration as a cause (Table 2). Inflammation is a common cause of fibrinogen elevation. However neither group had a differential leukocyte count that suggested inflammation. The increase was more severe in the pair fed group. Two factors may account for this. One, the pair fed dogs were 20 weeks older than high dosage dogs, and 2 they were on test 20 weeks later than the other dogs. The reason for the FIB elevation was not determined.

Elevated urine bilirubin was not an arsenic effect since no treated group had elevated levels. Because the pair fed group was tested later than the others and animals that had positive values were negative on other sampling dates and since there was no evidence of elevated serum bilirubin we concluded the elevation was not significant. For the same reasons the same conclusion was made regarding the pair fed group urine glucose elevation.

BUN of pair fed animals in phase IIa was lower than

other groups but within normal limits. In the same time period, serum creatinine was not significantly different between groups indicating that renal function was not the cause of the depression. Diminished protein intake can cause decreased BUN (Finco, 1980). Inadequate caloric intake causes increased protein catabolism and increased BUN (Finco, 1980; Duncan and Prasse, 1986). Considering these facts a possible senerio is that pair fed dogs were given a limited feed intake, therefore protein was limited and BUN decreased. However, high dosage dogs feed intake was the same as pair fed dogs but their BUN did not decrease. High dosage dogs were 20 weeks younger and had considerably less fat reserve than the pair fed dogs going into the trial. Therefore, high dosage dogs had to catabolize structural protein for energy. Hence, factors that caused decreased BUN and increased BUN may have neutralized each other. It is clear arsenic had no direct effect on BUN levels.

All serum albumin values were within normal limits, however significant differences were found amoung groups. In phase I serum albumin elevation was significant in medium dosage, high dosage and pair fed groups relative to controls. Kaneko (1980) states that true overproduction of albumin has not been known to occur in any animal. Since elevated values are still within the normal range, we conclude the difference is random variation. In phase IIa, high dosage and pair fed

groups had severely limited feed intake and significant decrease in serum albumin. Dietary malnutrition causes serum albumin depression (Duncan and Prasse, 1986). Since the pair fed group was not given arsenic, decreased serum albumin is not a direct effect of arsenic.

Urine ALP to CRT ratio elevations in the low dosage group was peculiar to that group and not an arsenic effect. This conclusion was reached because no other treated group had elevated values and a low dosage group pretrial urine sample had an elevated ratio (mean=4 IU/g).

Liver leakage enzymes AST and ALT were significantly elevated in serum of dogs treated with sodium arsenite. ALT values for the high dosage group were above the upper normal limit of 66 IU (Table 3). ALT is specific for the liver in dogs and is found normally free in the cytosol. AST is present in cytosol and mitochondria of many tissues (Cornelius, 1980). These enzymes are found in high concentrations within the normal cell. Serum levels increase when plasma membranes are altered and allow enzyme leakage (Duncan and Prasse, 1986).

Elevated serum enzymes in this study indicate that sodium arsenite causes altered hepatocyte plasma membrane permeability. Serum AST and ALT increases were low grade and consistent. Since AST and ALT have short serum half-lives

(1-3 days), this indicates hepatocyte damage was persistent (Duncan and Prasse, 1986).

Arsenic has an inhibitory effect on a multitude of enzymes, many of which could ultimately cause dysfunction of the plasma membrane (Webb, 1966). Reaction of arsenic with glutathione is a likely mechanism that would allow plasma membrane damage. Trivalent arsenic binds to many biological compounds which contain sulfhydryl groups. Glutathione reacts via sulfhydryl groups with cellular oxygen metabolites and lipid peroxides and prevents them from causing damage to the plasma membrane (Cheville, 1983). Binding of glutathione sulfhydryl groups to arsenic would effectively inhibit an important cellular antioxidation system. Pretreatment of animals with glutathione protected them from the toxic effects of arsenic (Voegtlin, 1925).

Canine exposure to sodium arsenite in the field causes an elevation of ALT (Evinger and Blakemore, 1984). However, serum AST was reduced in rats exposed to 50 mg/kg of arsenic as dietary arsenate (Mahaffey et al., 1981). This discrepancy may be due to the fact that rats and dogs metabolize arsenic differently (Vahter, 1983). Rats accumulate arsenic in the blood with a half-life of 60 to 90 days compared to a half-life of hours in the dog. This high blood arsenic in the rat may have interfered with measurement of serum enzymes.

Increased serum Na of low and medium dosage groups in phase I was not repeated by these or other groups in the rest of the experiment. This indicates that treatment was not the cause. Increased values were well within the normal range. The reason for these changes was not determined.

The increase of serum K in phase IIb of low and medium dosage groups was not repeated by these or other groups in the rest of the experiment. Increased serum K values were well within the normal range. It is doubtful this was a treatment effect, since no treated group in phase I or IIa had this change. However, treatment effect can not be ruled out. If it was a treatment effect, the pathophysiological mechanism for it is unknown.

The decrease of serum P in pair fed dogs was probably due to their age. Young animals have higher serum P values than adults (Duncan and Prasse, 1986). Pair fed dogs were 20 weeks older than the other dogs.

Phase I serum Ca elevation in medium dosage dogs and depression in pair fed dogs is unexplained. However, mean serum Ca of control and low dosage dogs decreased in each sequential phase (11.4, 11.2, and 11.0 mg/dl in phases I, IIa, and IIb respectively). Therefore the fact that pair fed dogs were 20 weeks older than the other dogs may explain why they had lower serum Ca values.

Serum Ca depression in high dosage and pair fed groups of phase IIa and medium dosage group in phase IIb could have been caused by decreased serum albumin (Duncan and Prasse, 1986). The medium group mean serum albumin of 3.7 g/dl was not statistically lower but considerably lower than that of the control group (4.5 g/dl).

In conclusion, dietary inorganic arsenic at these levels caused no renal dysfunction that was detectable by multiple parameters measured. This study indicates that dietary sodium arsenite causes low grade persistent damage to hepatocyte plasma membranes.

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TABLE 1

Blood, Serum, and Urine Sampling Schedule of Dogs Exposed to Dietary Sodium Arsenite

	Weeks on Trial									
Group	2	3	6	7	11	13	15	17	19	26
Control	X			X		X			X	X
Low dosage	X			X		X			X	X
Medium dosage	X			X		X			X	X
High dosage	X			X		X	X[a]		X[b]	
Pair fed		X	X		X			X		

[a]Two of 6 dogs.

[b]Four of 6 dogs.

TABLE 2

Mean Fibrinogen and Total Plasma Protein Values for Phase IIa of Dogs Exposed to Dietary Asenite

	Groups				
	Control	Low	Medium	High	Pair Fed
Fibrinogen (mg/dl)	133	150	158	192[a]	258[b]
Total Plasma Protein (g/dl)	6.3	6.3	6.5	5.9	6.3

[a]Significantly elevated relative to controls (p=0.05).

[b]Significantly different from all other groups (p=0.05).

TABLE 3

Mean Serum AST[a] and ALT[b] Values[c] of Dogs Exposed to Dietary Sodium Arsenite in Phases I, IIa, and IIb

	AST			ALT		
Group	I	IIa	IIb	I	IIa	IIb
Control	18	17	16	29	24	23
Low dosage	21	19	20	37	42	44*
Medium dosage	22	29**	29**	27	42	45**
High dosage	26*	26*		98**	84**	
Pair fed	16	15		23	22	

[a]Aspartate aminotransferase.

[b]Alanine aminotransferase.

[c]Given in IU/L.

*Statistically different than controls ($p<0.05$).

**Statistically different than controls ($p<0.01$).

SECTION III. EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON THE LIVER OF DOGS

EFFECT OF LOW LEVEL DIETARY SODIUM ARSENITE ON THE LIVER OF DOGS

(Effect of arsenite on canine liver)

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ABSTRACT

Thirty female beagle dogs, 7 to 8 months old, were assigned to 5 groups. Control, low dosage, medium dosage, high dosage, and pair fed groups were offered daily 0, 1, 2, 4, and 0 mg of dietary sodium arsenite per kg of body weight respectively. On day 59 the dosage was doubled for the rest of the experiment which ended on day 182. The pair fed group was offered the amount of feed that the high dosage group consumed on a percent body weight basis. High dosage and pair fed groups were terminated in week 17 because the high dosage group had lost a cumulative average of 20 percent of their original body weight. No gross or light microscopic lesions were present in any group. Hepatocyte glycogen content was abundant and not significantly different among control, medium dosage, high dosage, and pair fed groups. Hepatocyte lipid content was limited and not different between control and high dosage groups. Electron microscopic evaluation of control and high dosage group livers was done. Hepatocyte mitochondrial volume density was increased in the treated group by 22 percent. Individual mitochondrial perimeters and maximum diameters were significantly increased in the treated group. Mitochondrial circularity shape factor was significantly decreased in treated animals. This study shows that dietary sodium arsenite in dogs causes enlargement

of hepatocyte mitochondria. Evidence presented supports the conclusion that mitochondrial enlargement is arsenic induced swelling.

INTRODUCTION

Arsenic is ubiquitous in the environment and exposure is virtually impossible to avoid. In addition to natural sources the frequent use of arsenicals as herbicide and insecticidal bait creates the potential for accidental exposure to higher concentrations of arsenic. Arsenic has long been associated with liver damage (Soffer et al., 1937; Von Glahn et al., 1938; Finner and Calvery, 1939). There have been a few controlled studies of the effects of chronic low level arsenic exposure on dogs (Calvery et al., 1938; Byron et al., 1967). Significant inanition was produced at 125 ug/gm dietary arsenic as sodium arsenite or sodium arsenate (Byron et al., 1967). However, no gross or light microscopic liver changes were noted. Electron microscopic examination of liver was not done on dogs in the above mentioned studies.

Rats given access to drinking water containing 40 ug/gm of arsenic as sodium arsenate had a 1.2 fold increase in hepatocyte mitochondrial volume density (Fowler et al., 1979). Nuclei and vacuole volume densities were not changed and lysosome volume density was significantly decreased. Mice given access to drinking water with 85 ug/gm arsenic as sodium arsenate had no significant change in hepatocyte volume densities of mitochondria , nuclei, vacuoles , or

lysosomes (Fowler and Woods, 1979). Rats metabolize arsenic differently than other species (Vahter, 1983). To better understand the pathological mechanisms of arsenic it is important to know if hepatocyte intracellular organelles of other species react morphologically as they do in the rat or mouse.

Sodium arsenite is a trivalent arsenical that is relatively water soluble; therefore it is more toxic than most other arsenicals (Franke and Moxon, 1936; Harrisson et al., 1958; Savchuck et al., 1960). Sodium arsenite was selected for this study because of its high toxicity and its common occurrence in commercial chemicals and pesticides.

The purpose of this study was to evaluate the gross, light microscopic, and electron microscopic liver changes in dogs exposed to low level subchronic dietary inorganic arsenic.

MATERIALS AND METHODS

Thirty female beagle dogs, 7 to 8 months old, were randomly assigned to 5 groups (6 dogs per group). Control, low dosage, medium dosage, and high dosage groups were offered nominal dosages (intended dosage if dog consumed all feed offered to it) of 0, 1, 2, or 4 mg of sodium arsenite ($NaAsO_2$) per kilogram of body weight in the feed daily. After 58 days the dosage of all groups was doubled for the rest of the experiment. To account for substantial feed refusal in principals a pair fed group (inanition control) was fed the amount of feed, without sodium arsenite, that the high dosage group consumed on a percent body weight basis. Pairings were by group, not individual animals, and readjusted weekly to mimic intake of the high dosage group. The pair fed group was started on the experiment 20 weeks after the other groups. Dogs of the pair fed group were on average 20 weeks older than dogs of other groups.

Each dog of the control, low dosage, medium dosage, and high dosage groups was offered daily 2.75 percent of its body weight of dry dog food (Purina High Pro Dog Chow, Ralston Purina Co., St. Louis, Missouri) for the first 2 weeks, then 3.00 percent for the remainder of the experiment. At the feeding rate of 3 percent of body weight per day, initial nominal dosages are equivalent to 33.4, 66.7, and 133.4 ug of

sodium arsenite per gm of dry feed as fed for low, medium, and high dosage groups respectively. Uncontaminated feed samples were quantitated for arsenic by hydride atomic absorption spectroscopy (303 Perkin-Elmer Atomic Absorption Spectrometer, Perkin-Elmer MHS-10 Hydride System) according to methods previously described (Hyde et al., 1977). Feed contained less than 0.1 ug/gm arsenic.

Dry feed was mixed with an equal weight of tap water containing the appropriate dissolved dose of sodium arsenite. Feed and water were mixed thoroughly to insure even distribution of arsenic in feed. The amount of water added wet the feed evenly, and no excess water was left free of the feed. Feed was weighed and mixed with arsenic every morning. The mixture was offered to each dog for 6 to 8 hours. Each night, unconsumed feed was weighed, recorded, and discarded. The actual arsenic dosages were calculated daily by multiplying the percent of offered feed consumed by the appropriate nominal dosage. Body weights were determined and the amount of feed and arsenic adjusted weekly. The dogs had free choice tap water at all times. Tap water contained less than 0.01 ppm of arsenic. Feed and water were offered in stainless steel bowls.

The dogs were housed individually in stainless steel cages (Shor-Line, Shroer Manufacturing Co., Kansas City, Missouri). Each unit consisted of 2 cages, one above the

other. The assignment of dogs to cages was random except the bottom dog was always from the same group as the top dog. The dogs were observed at least 3 times a day, and the cages cleaned daily.

At the end of week 17, high dosage and pair fed groups were terminated because the high dosage group dogs had lost approximately 20 percent their original body weight. Control, low dosage, and medium dosage groups were terminated after 26 weeks.

Livers of all dogs were evaluated grossly and with the light microscope. Control and high dosage groups were also examined with the electron microscope.

Dogs were anesthetized with pentobarbital. A small (5-6 cm^3) specimen was collected from deep in the right medial lobe of the liver and immersed in 10% neutral buffered formalin. For control and high dosage group animals, part of the specimen was diced to approximately $1mm^3$ and immersed in 3 % glutaraldehyde/0.1M cacodylate buffer at pH 7.2. Animals were then euthanized with an overdose of pentobarbital and a complete postmortem was performed. The liver was examined grossly and weighed after nonhepatic tissue was removed.

Liver for light microscopic examination was taken from 10% neutral buffered formalin, processed by standard paraffin techniques (AFIP, 1960), sectioned at 5 um, and stained with

hematoxylin and eosin. To evaluate glycogen content, two paraffin sections from each dog of control, medium dosage, high dosage, and pair fed groups were made. One section from each dog was stained with periodic acid Schiff (PAS). The other section was treated with diastase and then stained with PAS. To evaluate lipid content, frozen sections of formalin fixed tissue of each dog in the control and high dosage groups were stained with oil red O. All light microscopic evaluation was done without knowledge of treatment group. Slides evaluated for glycogen and fat content were graded on a scale of 1 to 10 in ascending order as content increased.

Tissue saved for electron microscopic evaluation was removed from the glutaraldehyde/cacodylate buffer after one hour and rinsed in pH 7.2 cacodylate buffer (0.1M). Then it was post fixed in 1% Osmium tetraoxide/0.1M cacodylate buffer for one hour. After several rinses in distilled water the specimens were dehydrated in acetone, infiltrated with acetone resin, dried, embedded and cured. Three randomly selected blocks of embedded tissue from each dog were sectioned (60-90 nm thick) and stained with 2% methanolic uranyl acetate and Reynolds' lead citrate (Reynolds, 1963). With the use of a transmission electron microscopic (HS-9, Hitachi Ltd., Tokyo, Japan) the upper left corner of the first three upper left complete grid spaces that contained hepatocytes were photographed. Final magnification of the

electron micrographs was 15,890. Total number of micrographs per dog was 9.

Stereological methods were used to compare liver ultrastructure of control and high dosage groups. Profiles of the ultrastructure of the hepatocytes were traced onto transparencies which were analyzed by a computerized image analysis system (Zeiss SEM-IPS Image Analysis System, Carl Zeiss Inc., Thornwood, New York). Volume and surface densities of nuclei, fat globules, mitochondria, and lysosomes (primary and secondary) were measured (Weibel, 1979). Volume density is the relative volume of an object of interest within a given reference volume. Surface density is the surface area of an object of interest within a corresponding reference volume. In this study, objects of interest were nuclei, fat globules, mitochondria, and lysosomes. Reference volume in all cases was hepatocyte cell volume. The formula for volume density (V_D) is:

$$V_D = A / RefA$$

where A = sum of area of profiles of interest and RefA = reference area. The surface density (S_D) formula is:

$$S_D = 4P / 3.14RefA$$

where P = sum of perimeter of profiles of interest. V_D and S_D were calculated with data summed over electron micrographs from blocks of tissue. Calculated V_D and S_D values were

statistically analyzed as repeated measures from the experimental unit (dog). The number of mitochondrial profiles per reference area was counted.

Two dimensional characteristics of individual hepatocyte mitochondria of control and high dosage groups were measured. Measurements were made from the transparencies made for the stereological procedure. Measurements included area, perimeter, circularity shape factor, equivalent circular diameter, maximum diameter, and minimum diameter (Schwarz, 1980). Circularity shape factor (Cirsf) of a circle is 1. As the shape of an object becomes elongate or more irregular the Cirsf gets progressively less. The formula for Cirsf is:

$$\text{Cirsf} = 4\ (3.14)\ (\text{Ap})/\text{OPp}^2$$

where Ap = area of an individual mitochondrial profile and OPp = outside perimeter of an individual mitochondria profile. Equivalent circular diameter (Ecd) is the diameter of a circle that has the same area as a mitochondrion being measured. The formula for Ecd is:

$$\text{Ecd} = 2\ (\text{Ap}/3.14)^{1/2}$$

The two dimensional characteristics calculated for each mitochondria were averaged within each electron micrograph and analyzed as repeated measure of experimental unit (dog).

The experiment was of complete random design used to compare groups of different treatments. Individual dogs were the experimental units. Analysis of variance was done

utilizing the computer based Statistical Analysis Systems (SAS Institute Inc., Cary, North Carolina). Least significant differences were calculated from the experimental error of dogs within groups (Snedecor and Cochran, 1967).

RESULTS

The average daily sodium arsenite consumption per kg of body weight is shown in Table 1. Arsenite caused a statistically significant dosage dependent decrease in body weight. This decrease is described in more detail elsewhere (Neiger and Osweiler, 1987a). Serum enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) but not alkaline phosphatase were significantly elevated by arsenite exposure, also described elsewhere (Neiger and Osweiler, 1987b).

None of the livers had gross lesions at post-mortem examination. Liver weight per body weight of medium dosage group was significantly different from that of controls. Mean liver weight per body weight for control, low dosage, medium dosage, high dosage, and pair fed groups were 29, 27, 33, 31, and 29 gm/kg respectively. The least significant difference between groups was 3.8 ($p=0.05$). Therefore only the medium dosage group differed significantly from the control group.

Light microscopy revealed no difference amoung groups. No lesions were found in any group on evaluation of hematoxylin and eosin stained sections. Hepatocyte glycogen content was abundant and not significantly different among control, medium dosage, high dosage, and pair fed groups. Hepatocyte lipid content was limited and not different

between control and high dosage groups.

Hepatocyte mitochondrial volume density was significantly increased in the high dosage group (Table 2). However, the mitochondrial surface density was not statistically changed by arsenite exposure. Volume and surface densities of the hepatocyte nuclei, lysosomes, and fat globules did not differ significantly between control and high dosage groups (Tables 2 and 3). The number of mitochondrial profiles per reference area was not significantly different ($p=0.72$) between control and high dosage groups and were 0.25 and 0.24/um^2 respectively.

Three of the 2 dimensional characteristics of individual hepatocyte mitochondria were significantly changed by the treatment (Table 4). The perimeter and maximum diameter of mitochondria from the high dosage group were significantly increased ($p=0.03$ and 0.01 respectively). Circularity shape factor was significantly lowered in the high dosage group ($p=0.01$). Minimum diameter between groups was not statistically different ($p=0.27$). The area per mitochondria and equivalant circular factor were both increased in the high dosage group but not significantly ($p=0.10$).

DISCUSSION

Serum enzymes AST and ALT were significantly elevated in high and medium dosage groups but not in the pair fed group (Neiger and Osweiler, 1987b). This gives evidence there is a direct effect of arsenic on the liver. All changes in treated groups must be interpreted in light of a highly significant dosage dependent decrease in feed intake and body weight (Neiger and Osweiler, 1987a). The high dosage group dogs lost, on average, approximately 20 percent of their initial body weight.

Increase of medium dosage group liver weight per body weight was not paralleled by any other group leaving the cause of the increase undetermined. High dosage and pair fed groups had highly significant weight loss and no significant change in the liver weight to body weight ratio. Therefore, the liver weight loss was proportional to the body weight loss. Decrease of liver reserves is expected in chronically starved animals (Kelly, 1985).

Mitochondrial volume density was increased and was the only sterologically measured parameter changed by arsenic treatment (Table 2). Increased mitochondrial numbers or size or both could cause this change. No change in the number of mitochondrial profiles per reference area suggests that the number of mitochondria per volume did not increase.

The ratios of number of mitochondria per hepatocyte volume and mean mitochondrial volume between high dosage and control groups can be approximated using the concept of diameters of equivalent spheres (Weibel, 1979). The diameter of an equivalent sphere is the diameter of a sphere with the same volume as the object of interest (mitochondria). There is some variation of the shape of mitochondria in this study, however the majority appear to be prolate ellipsoides. The average length to width ratio is less than 2 as estimated by the ratio of maximum diameter to minimum diameter. In the case of prolate ellipsoides with an eccentricity of 2, the difference between equivalent diameter and true mean diameter is only 9 percent (Weibel, 1979). Therefore, the error incurred by using diameters of equivalent spheres is less than 9 percent.

The development of the calculations of the number of mitochondria per volume and mean mitochondrial volume ratios is as follows. The mean volume of a mitochondrion ($\bar{V}$) equals its volume density (V_D) divided by its numerical density (number of mitochondria per hepatocyte volume, N_D).

(1) $$\bar{V} = V_D / N_D$$

It has been proven that numerical density of an object is the number of objects per area (N_A) divided by the mean caliper diameter ($\bar{D}$) of the object(Weibel, 1979).

(2) $$N_D = N_A / \bar{D}$$

In this case the mean caliper diameter is the diameter of an equivalent sphere of the mitochondria. Using equations 1 and 2 the ratio between mean mitochondrial volumes can be expressed.

(3) $$\bar{V}_c / \bar{V}_h = (V_{Dc}/V_{Dh}) \ (N_{Dh}/N_{Dc})$$

The subscript c indicates control group and h indicates high dosage group. Equation 2 can be substituted into equation 3 to yield:

(4) $$\bar{V}_c / \bar{V}_h = (V_{Dc}/V_{Dh}) \ (N_{Ah}/N_{Ac}) \ (\bar{D}_c/\bar{D}_h)$$

where V_D and N_A are known. The volume of a sphere is:

(5) $$V = 1/6 \ (3.14) \ D^3$$

Substituting equation 5 into equation 4 yields:

(6) $$1/6(3.14)D^3_c/1/6(3.14)D^3_h = (V_{Dc}/V_{Dh}) \ (N_{Ah}/N_{Ac}) \ (\bar{D}_c/\bar{D}_h)$$

This equation can be solved for the ratio of the mean diameter of equivalent spheres of mitochondria for control and high dosage animals.

(7) $$\bar{D}_c / \bar{D}_h = [(V_{Dc}/V_{Dh}) \ (N_{Ah}/N_{Ac})]^{1/2}$$

Using data from this study, the evaluation of this results in:

(8) $$\bar{D}_c / \bar{D}_h = [(0.139/0.169) \ (0.24/0.25)]^{1/2} = 0.889$$

From equation 7 and equation 2 the ratio of numerical densities can be found.

(9) $$(N_{Dc}/N_{Dh}) = (N_{Ac}/N_{Ah}) \ (\bar{D}_h/\bar{D}_c)$$

Using data from this study, the evaluation of this results

in:

(10) $(N_{Dc}/N_{Dh}) = (0.25/0.24)\ (1/0.889) = 1.172$

The ratio of mean mitochondrial volumes of the control versus high dosage dogs can be found using equation 4.

(11) $\bar{V}_c / \bar{V}_h = (0.139/0.169)\ (0.24/0.25)\ (0.889) = 0.702$

The mitochondrial numerical density ratio of 1.172 indicates the number of mitochondria per hepatocytic volume remained the same or slightly decreased in dogs treated with arsenic. The ratio of mean mitochondrial volumes of 0.702 indicates dogs treated with arsenic had enlarged mitochondria.

Increases of mean area, perimeter, and maximum diameter of individual mitochondria also support the supposition that increased mitochondrial volume density is due to increased mitochondrial size (Table 4). However, the mean mitochondrial area increase of the high dosage group was not statistically significant with a p value of 0.11. Significant decrease in the mean circularity factor and increase of the mean maximum diameter suggest the mitochondria became elongated (Table 4).

Two main ways mitochondria enlarge are by swelling or hypertrophy. Swelling involves the entry of solutes and water due to a defect in the osmotic homeostatic mechanisms (Cheville, 1983). Lack of ATP is a major cause of mitochondrial swelling.

Inorganic arsenic as arsenite (trivalent) or arsenate

(pentavalent) can inhibit ATP production. Vahter (1983) reveiwed the literature and suggested that trivalent or pentavalent inorganic arsenic can be interconverted by in vivo metabolism. Rats injected with arsenite have the same hepatocytic mitochondrial metabolic alteration as rats given oral arsenate (Ghafghazi et al., 1980). Arsenate uncouples electron transport in the mitochondrial respiratory chain from oxidative phosphorylation (Squibb and Fowler, 1983). It also inhibits pyruvate dehydrogenase and/or its regulating enzymes presumably by binding to a critical phosphate binding site (Schiller et al., 1977; Schiller et al., 1978). Trivalent inorganic arsenicals like arsenite inhibit enzymes by binding to their sulfhydryl groups (Squibb and Fowler, 1983). In mitochondria the critical enzyme pyruvate dehydrogenase is susceptible to inactivation by trivalent arsenic. Therefore, reduced amounts of ATP in treated animals is likely and could cause mitochondrial swelling.

Mitochondrial hypertrophy develops in response to demands for energy, starvation or deficits of critical substrates, vitamins, and minerals (Cheville, 1983). Megamitochondria and/or reduplication and elongation of cristae are associated with hypertrophic mitochondria (Tandler and Hoppel, 1980; Riede et al., 1973). These changes were not obviously present in mitochondria studied.

However, more sensitive sterological techniques were not used to quantify mitochondrial internal membranes. Therefore a subtle change associated with hypertrophy cannot be ruled out. However, because of the lack of megamitochondria and/or reduplication or elongation of cristae, we conclude that mitochondrial enlargement is due to arsenic induced mitochondrial swelling. Evaluation of the pair fed group hepatocyte mitochondria should be done to confirm this conclusion.

Starvation depletes liver stores of carbohydrates (Cheville, 1983; Kelly, 1985). In this study liver glycogen was highly conserved in all groups evaluated. The pair fed group dogs may have been better able to conserve the liver glycogen because they were older and weighed more. However, the high dosage group was very thin at termination and the presence of normal amounts of liver glycogen was unexpected. It is possible the glycogen was conserved in the high dosage group because of the blocking of its entry into the citric acid cycle by inactivation of pyruvate dehydrogenase by the arsenic. Another explanation would be if the high dosage and pair fed dogs converted primarily to lipid and ketone energy metabolism. This would allow what little glucose was taken in daily to be stored as glycogen.

In conclusion liver mitochondria of dogs exposed to dietary arsenic were enlarged, elongated and had a greater

volume density than controls. Evidence presented supports the conclusion that mitochondrial enlargement is caused by arsenic induced swelling. These changes are similar to those found in rats exposed to sodium arsenate (Fowler et al., 1979).

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TABLE 1

Average Daily Sodium Arsenite Dosage[a]

in Dogs Offered Contaminated Feed

	Group[b]				
	Control	Low	Medium	High	Pair Fed
Weeks 1-8	0.0	0.88	1.80	2.88	0.0
Weeks 9-17	0.0	1.66	2.87	3.74	0.0
Weeks 9-26	0.0	1.59	3.00		

[a]Dosage of sodium arsenite consumed in mg/kg of body weight.

[b]Six dogs in each group.

TABLE 2

Mean Volume Densities[a] of Hepatocyte Ultrastructural Features of Dogs Offered Sodium Arsenite Contaminated Feed

	Control group	High dosage group	Pr>F[b]
Nuclei	0.041	0.061	0.30
Fat globules	0.001	0.003	0.37
Mitochondria	0.139	0.169	0.04
Lysosomes	0.005	0.004	0.14

[a]Units are um^3/um^3.

[b]Probability of a greater F value as determined by analysis of variance.

TABLE 3

Mean Surface Densities[a] of Hepatocyte Ultrastructural Features of Dogs Offered Sodium Arsenite Contaminated Feed

	Control group	High dosage group	Pr>F[b]
Nuclei	0.059	0.079	0.40
Fat globules	0.007	0.016	0.26
Mitochondria	1.005	1.119	0.28
Lysosomes	0.037	0.030	0.33

[a]Units are um^2/um^3.

[b]Probability of a greater F value as determined by analysis of variance.

TABLE 4

Mean Area, Perimeter, and Shape Indices of Individual Hepatocyte Mitochondria of Dogs Offered Sodium Arsenite Contaminated Feed

	Control group	High dosage group	Pr>F[a]
Area (um^2)	0.55	0.70	0.11
Perimeter (um)	2.86	3.30	0.03
Circularity shape factor	0.76	0.74	0.01
Maximum diameter (um)	1.01	1.19	0.01
Minimum diameter (um)	0.67	0.72	0.27
Equivalent circular diameter (um)	0.78	0.87	0.10

[a]Probability of a greater F value as determined by analysis of variance.

SUMMARY AND DISCUSSION

Dietary sodium arsenite causes a dose dependent decrease of feed consumption and body weight in dogs. Nominal dosages of 4 and 8 mg of sodium arsenite per kg of body weight per day (mg/kg/day) caused the decreases. After initial decreased feed consumption, feed intake slowly increased in all cases over time. Weight loss has been demonstrated previously in pigs, mice, rats, and dogs exposed to inorganic arsenic (Calvery et al., 1938; Byron et al., 1967; Fowler and Woods 1979; Morrison and Chavez, 1983).

Nominal dosages of 1 and 2 mg/kg/day did not affect feed consumption or body weight. Dogs pair fed an amount of control feed that the high dosage group consumed also lost weight. Regression analysis of body weight demonstrated no significant difference between these groups. This proved weight loss is due to decreased feed consumption rather than a direct effect of arsenic.

Serum liver leakage enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were elevated in certain treated groups relative to controls. AST was elevated in dogs exposed to 4 and 8 mg/kg/day of sodium arsenite. ALT was elevated by a short term (6 weeks) exposure to 4 and 8 mg/kg/day and longer exposure (25 weeks) to 2 mg/kg/day. ALT and AST elevations relative to controls

were persistent and low grade. Only ALT in the high dosage group was elevated above what is considered normal as determined by Duncan and Prasse (1986). Elevated serum ALT and AST indicates sodium arsenite causes altered hepatocyte plasma membrane permeability. Accidental exposure of a dog to sodium arsenite in the field causes elevation of ALT (Evinger and Blakemore, 1984).

The liver was examined grossly and with the use of light and electron microscopy. No gross or light microscopic lesions were present in any group. Electron microscopic examination of control group and high dosage group livers revealed a 22 percent increase of mitochondrial volume density in the treated group. Individual mitochondrial perimeters and maximum diameters were significantly increased in the high dosage group. Mitochondrial circularity shape factor was significantly decreased in treated animals. This study shows that dietary sodium arsenite in dogs causes enlargement of hepatocyte mitochondria. Evidence in this study supports the conclusion that the enlargement is arsenic induced swelling. Arsenic induced hepatocytic mitochondrial swelling has also been described in rats (Fowler et al., 1979b).

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APPENDIX A. ANIMAL RESEARCH FORM

APPLICATION ON THE USE OF ANIMALS IN RESEARCH AND TEACHING

IOWA STATE UNIVERSITY

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If your teaching or research involves the use of any warm-blooded animals (other than Homo sapiens), please submit two copies of this completed form at the time that the research proposal is submitted or at least 30 days before any animals are used in teaching or in-house research. One copy of your research proposal or a brief description of the animal use in teaching should be sent to Ronald E. Flatt, D.V.M., 1426 Vet. Med. Dr. Flatt will return one copy of the signed form to you and keep one copy for his files.

Name of Investigator or Course Instructor Gary D. Osweiler

Department Veterinary Diagnostic Laboratory

Title of Research Proposal (Research) or Course Name and Number (Teaching)

Effects of Arsenic in Dogs

If animals are to be housed overnight or longer, the animal facilities must be inspected by the Laboratory Animal Facilities Committee. Please identify facilities where the animals will be housed.

Building _____ Room No. _____

What animals do you intend to use? Research Beagles

If any procedure causes more pain than injection of an anesthetic into an animal, then an anesthetic should be used.* Is pain involved in your proposed use of animals?

Yes _____ No X

If yes, identify anesthetic you propose to use: _____

Dose of anesthetic: _____

If the animals are to be killed, identify the method of euthanasia: _____

T-61 Euthanasia Solution

*In the event that anesthetizing the animal(s) will interfere with results of your proposed animal use, you may elect not to use an anesthetic. If you decide not to use an anesthetic, please attach your justification for not using appropriate anesthetics when pain is involved.

Laury L. G. Lenton — Department Executive Officer — Date

Gary D. Osweiler — Investigator/Instructor — 8-2-83 Date

(FOR OFFICE USE ONLY)

Facilities: [X] Approved [ ] Disapproved

Protocol: [X] Approved [ ] Disapproved

Comments: _____

Ronald E. Flatt — Ronald E. Flatt, D.V.M., Ph.D. — 12 Aug 83 Date

APPENDIX B. CLINICAL PATHOLOGY DATA

TABLE B1

Serum Chemistry Values of Dogs

Exposed to Dietary Sodium Arsenite

	Phase	BUN[a]	CRT	GLU	TB	DB	AST[b]	ALT	ALP	ALB[c]
Mean[d]	I	14	0.7	92	0.1	0.1	20	43	36	3.9
S.E.[e]	I	3.4	0.1	6	0	0	5*	44*	13	0.2*
Mean	IIa	15	0.8	90	0.1	0.1	21	43	31	4.1
S.E.	IIa	2.4**	0.1	8	0	0	7**	22**	20	0.4
Mean	IIb	15	0.8	91	0.1	0.1	22	37	31	4.1
S.E.	IIb	3.5	0.1	13	0	0	11**	22*	43	0.6

[a]Values for BUN, CRT, GLU, TB, and DB are expressed in mg/dl.

[b]Values for AST, ALT, and ALP are expressed in IU/l.

[c]Values for ALB are given in gm/dl.

[d]Overall mean.

[e]Standard error = square root of mean square of dogs within groups.

*Probability of statistically significant differences among groups is greater than 95 percent.

**Probability of statistically significant differences among groups is greater than 99 percent.

TABLE B2

Serum Electrolyte Values of Dogs

Exposed to Dietary Sodium Arsenite

		Serum					Urine			
	Phase	NA[a]	K	CA[b]	P	CL	NA	K	CL	P
Mean[c]	I	148	4.4	11	6.0	108	103	105	55	115
S.E.[d]	I	1.0*	0.2	0.2**	0.5**	1.4	49	37	27*	56
Mean	IIa	146	4.4	11	5.2	110	41	116	21	115
S.E.	IIa	2.6	0.3	0.6**	0.7**	4.3	17**	55	18*	56
Mean	IIb	146	4.4	11	5.2	111	62	107	35	91
S.E.	IIb	1.6	0.3*	0.7*	0.8	2.2	49	42	31*	59

[a]Values for Na, K, and Cl are expressed in mEq/L.

[b]Values for Ca and P are expressed in mg/dl.

[c]Overall mean.

[d]Standard error = square root of mean square of dogs within groups.

*Probability of statistically significant differences among groups is greater than 95 percent.

**Probability of statistically significant differences among groups is greater than 99 percent.

TABLE B3

NA, K, P, and CL Renal Percent Clearance Ratios[a] of Dogs Exposed to Dietary Sodium Arsenite[b]

	Phase	PCRNA	PCRK	PCRP	PCRCL
Mean[c]	I	0.33	10.7	8.5	0.23
S.E.[d]	I	0.19	4.4	3.9	0.13
Mean	IIa	0.14	12.8	10.5	0.09
S.E.	IIa	0.09	8.2	5.8	0.10
Mean	IIb	0.29	14.1	9.3	0.22
S.E.	IIb	0.30	8.7	6.5	0.26

[a]PCR

[b]No statistically significant differences were present among groups in any phase.

[c]Overall mean.

[d]Standard error = square root of mean square of dogs within groups.

TABLE B4

Hematological Values of Dogs

Exposed to Dietary Sodium Arsenite

	Phase	HGB (gm/dl)	PCV (%)	FIB (mg/dl)	TPP (gm/dl)
Mean[a]	I	16.7	48	133	6.3
S.E.[b]	I	1.1	3	29	0.2
Mean	IIa	16.1	47	178	6.3
S.E.	IIa	1.3	4	49**	0.3
Mean	IIb	15.6	45	167	6.4
S.E.	IIb	2.2	6	32	0.5

[a]Overall mean.

[b]Standard error = square root of mean square of dogs within groups.

**Probability of statistically significant differences among groups is greater than 99 percent.

TABLE B5

White Blood Cell Numbers of Dogs

Exposed to Dietary Sodium Arsenite[a]

	Phase I		Phase IIa		Phase IIb	
	Mean[b]	S.E.[c]	Mean	S.E.	Mean	S.E.
Total	9008[d]	1991	8503	2308	8865	3763
Band neutrophils	182	99	164	105	161	148
Segmented neutrophils	5718	1734	5665	2112	6030	3445
Lymphocytes	2564	643	2247	393	2270	612
Monocytes	283	165	167	143	167	232
Eosinophils	261	187	260	177	256	321

[a]No statistically significant differences were present among groups in any phase.

[b]Overall mean.

[c]Standard error = square root of mean square of dogs within groups.

[d]Values given in number of cells per ul.

TABLE B6

Urinalysis of Dogs

Exposed to Dietary Sodium Arsenite

	Phase I		Phase IIa		Phase IIb	
	Mean[a]	S.E.[b]	Mean	S.E.	Mean	S.E.
Specific gravity	1.035	0.023	1.031	0.010	1.030	0.010
Osmolality (mOsm/l)	1112	333	1114	365	1014	346
Urobilinogen (Ehrlich unit/dl)	0.74	0.29	0.58	0.35	0.41	0.42
Blood[c]	0.01	0.02	0.13	0.41	0.13	0.47
Bilirubin	0.09	0.22*	0.04	0.24	0.09	0.27
Ketones (mg/dl)	0.26	0.80	0.89	1.50	0.91	1.90
Glucose (gm/dl)	0.00	NA[d]	0.01	0.01**	0.00	NA
pH	7.35	0.66	7.03	0.68	7.31	0.69
Protein (mg/dl)	11.6	14.4	14.7	13.3	14.4	23.0

[a]Overall mean.

[b]Standard error = square root of mean square of dogs within groups.

[c]Blood and bilirubin were measured with a scale of 0, 0.1, 1, 2, and 3 representing negative, trace, small, moderate, and large amounts respectively.

[d]Not applicable.

APPENDIX C. PHOTOMICROGRAPH OF HEPATOCYTIC MITOCHONDRIA

Fig. C1. Photomicrograph of hepatocytic mitochondria from a dog of the control group. 15,890 X.

Fig. C2. Photomicrograph of hepatocytic mitochondria from dog of the high dosage group. 15,890 X.